Proceedings
of the
Society
for
Experimental Biology and Medicine

Vol. 43. April, 1940. No. 4.

SECTION MEETINGS

CLEVELAND		
Western Reserve University	April	12, 1940
ILLINOIS		
University of Chicago	April	9, 1940
MINNESOTA		
University of Minnesota	March	20, 1940
MISSOURI		
Washington University	April	10, 1940
PACIFIC COAST		
Mount Zion Hospital, San Francisco	March	8, 1940
PEIPING		
Peiping Union Medical College	February	7, 1940

11276 P

Effect of Meat Extract on Fatty Infiltration of Liver in Depancreatized and Duct-Ligated Dogs

Elaine P. Ralli and Saul H. Rubin.

From the Laboratories of the Department of Medicine, New York University College of Medicine.

Since Fisher[1] and Allan, *et al.*,[2] first reported fatty infiltration of the livers in depancreatized dogs maintained on insulin, the observation has been confirmed repeatedly.[3] Recently, it has been shown in this[4] and other[5, 6] laboratories that ligation of the external pancreatic ducts results in similar fatty changes in the liver. In all of these studies the diet fed to the dogs consisted largely of raw whole meat and glucose.

---

[1] Fisher, N. F., *Am. J. Physiol.*, 1924, **67**, 634.

[2] Allan, F. N., Bowie, D. J., MacLeod, J. J. R., and Robinson, W. L., *Brit. J. Exp. Path.*, 1924, **5**, 75.

[3] Best, C. H., and Ridout, J. H., in Luck, J. M., *Ann. Rev. Biochem.*, 1939, **8**, 349.

In the experiments reported here a dried meat powder preparation,* from which the extractives had been removed, was substituted for the whole meat. The diet contained meat powder, cracker meal, milk powder, salts, brewer's yeast, bone ash, cod liver and corn oil. As shown in Table I, little or no fatty infiltration of the liver occurred in 6 depancreatized and in 3 duct-ligated dogs which were fed the diet for periods varying from 6.5 to 19 weeks after operation. The fatty acid content of the whole livers, determined as previously described,[7] averaged 6.2% with a range of 3.12 to 10.5% (Table I). When the meat powder diet was supplemented with 15 ml of a concentrated meat extract* (the combination of meat powder plus extract being equivalent to about 250 g of raw lean beef) the livers of

TABLE I.
Liver Lipids of Depancreatized and Duct-Ligated Dogs Fed Meat Powder or Meat Powder Plus Meat Extract.

Dog No.	Condition of animal*	Meat supplement†		Time after operation, wks	Body wt		Liver		
		Powder, g/day	Extr., ml/day		Initial, kg	Final, kg	Wt, g	Fatty acids, %	Total cholesterol, %
180	D	35	0	6.5	10.3	8.0	358	3.1	.27
176	D	35	0	12	8.0	6.4	232	10.4	.33
192	D	35	0	12	15.8	10.0	470	9.0	.23
196	D	50	0	11	13.0	12.3		3.4	.22
170	D	50	0	13	13.3	12.8	416	3.2	.23
198	D	75	0	19	10.3	11.3	403	4.4	.24
147	L	35	0	14	13.0	7.4	212	10.5	.35
193	L	35	0	13.5	11.8	10.0	273	3.7	.29
189	L	50	0	12	11.8	9.8	267	8.2	.32
216	D	50	15	5	9.8	6.8	377	19.5	.25
214	D	75	15	7	9.8	7.0	456	18.3	.29
203	L	50	15	9	9.3	6.8	294	19.8	.23

* D = depancreatized; L = duct-ligated dog.
†60 g meat powder + 15 ml extract ≎ 250 g whole meat.

---

[4] Ralli, E. P., Rubin, S. H., and Present, C. H., *Am. J. Physiol.*, 1938, **122**, 43.

[5] Person, E. C., Jr., and Glenn, F., *Arch. Surg.*, 1939, **39**, 530.

[6] Montgomery, M. L., Entenman, C., and Chaikoff, I. L., *J. Biol. Chem.*, 1939, **128**, 387.

* Dried meat powder and concentrated meat extract were purchased from the Valentine Meat Juice Company, Richmond, Va. The meat extract is a hot water extract of lean beef. It contains 4.5% proteose and peptone after it has been concentrated. This concentrated extract is what was used. Practically all of the protein and fat of the beef is in the powder.

[7] Rubin, S. H., Present, C. H., and Ralli, E. P., *J. Biol. Chem.*, 1937, **121**, 19.

one duct-ligated and 2 depancreatized dogs contained 18.3 to 19.8% fatty acids after 5 to 9 weeks (Table I). It is therefore concluded that the fatty liver of these dogs is due in large measure to some substance or substances present in the extractive fraction of meat.

11277

Analysis of the Bile of the Capuchin Monkey.

FRANCES F. BECK. (Introduced by John C. Krantz, Jr.)
From the Department of Pharmacology, School of Medicine, University of Maryland.

Studies in this laboratory in recent years have been concerned with the hydrogen-ion concentration of the bile of the guinea pig[1] and dog[2] with respect to the dissolution of human gall stones. Having available a series of Capuchin monkeys *(Cebus fatuellus)* upon which metabolism studies had been conducted, it was deemed of interest to study the bile of this species of monkey. The animals were narcotized with sodium amytal; the bile was drawn from the gall bladder without exposure to air. The hydrogen-ion concentration was determined by means of a glass electrode at 25° and an analysis of the principal constituents was carried out according to the Douglas-Sauermann[3] method. The results on 10 animals are shown in Table I.

The average pH of the bile and the percentage of various constituents present in the Capuchin monkey are similar to these data on the gall-bladder bile of dogs.[2] Also as with the bile of the dog it was observed that the correlation between a high pH and high total solids content is great.

TABLE I.
Constituents of Monkey Gall Bladder Bile.

	pH	Total solids, %	Mucin, %	Ash, %	Alkalinity of ash as % Na_2CO_3 in bile	Lipoids, %	Bile acids as cholic and desoxycholic, %
Aver.	6.3	20.8	0.81	1.65	0.65	7.95	6.05
Low	5.6	10.3	0.46	1.04	0.26	3.81	4.30
High	6.9	28.8	1.15	2.06	0.99	11.30	10.00

---

[1] Krantz, J. C., Jr., Feldman, M., Morrison, S., and Carr, C. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 48.

[2] Beck, F. F., Krantz, J. C., Jr., Feldman, M., and Carr, C. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 357.

[3] Douglas-Sauermann, A. G., *Z. Physiol. Chem.*, 1935, **231**, 92.

11278

Nature of the Urinary Androgens of Castrate Men.

WALTER HOSKINS* AND BRUCE WEBSTER.

From the Barbara Henry Research Laboratory of the New York Hospital and Department of Medicine, Cornell University Medical College, New York City.

A number of investigators have reported small amounts of urinary androgens in human male castrates[1-5] but the nature of the active material has not been established. A comparison of this androgenic material with that from the urine of normal men is of interest in view of the increasing use of urinary androgen values as measures of testis activity.

The present report describes the measurement of the androgens in the urine of male castrates and the fractionation of this material with digitonin in an effort to characterize the androgens present.

A complete urine collection was made from two male surgical castrates for 9 and 7 days respectively and the urines pooled. One subject had been castrated 10 years previously at the age of 37, the other 35 years before at the age of 26. The urine was kept in the refrigerator without added preservative and used within 3 days after its collection. Acid hydrolysis, benzene extraction and separation into androgenic and estrogenic fractions were carried out according to the methods of Gallagher, *et al.*[6] The androgenic fraction was taken up in 60% alcohol and assayed by inunction on the capon comb in parallel with pure androsterone as a standard.† Assays on groups of 4 to 6 capons showed an androgen excretion of 6 international units per day.

One-half of the androgenic fraction, the equivalent of 8 days' excretion, was treated with digitonin to separate any dehydroisoandrosterone (or other sterols having an hydroxyl group at position 3 in the beta configuration) as the insoluble digitonide.[7] The

---

* General Education Board and Barbara Henry Research Fellow.

[1] Koch, F. C., *J. Urology*, 1936, **35**, 382.

[2] Chou, C. Y., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 429.

[3] Callow, R. K., *Proc. Roy. Soc. Med.*, 1938, **31**, 841.

[4] Hansen, E. H., *Endokrinologie*, 1938, **21**, 9.

[5] McCullagh, E. P., *J. A. M. A.*, 1939, **112**, 1037.

[6] Gallagher, T. F., Peterson, D. H., Dorfman, R. I., Kenyon, A. T., and Koch, F. C., *J. Clin. Invest.*, 1937, **16**, 695.

† We are grateful to the Ciba Corporation, Lafayette Park, New Jersey, for the androgens used and to Robert Kinloch for his assistance with the assays.

[7] Butenandt, A., Dannenbaum, H., Hanisch, G., and Kudszus, H., *Z. Physiol. Chem.*, 1935, **237**, 57.

TABLE I.
Androgen Content of Urine-fractions of Normal and Castrate Men.

		Digitonin-treated urine extracts							
		Androsterone		Dehydroisoandrosterone					
				γ/day					
	Untreated urine (I.U./day)	γ/day	I.U./day	*Obs.*	*Corr.*	I.U./day	% A.‡ by wt	% D.§ by wt	Ratio A. to D. by wt
Normal	42	2925	29.25	1400	2000*	6.25†	59	41	3:2
Castrate	6	450	4.5	70	100*	0.3†	82	18	4:1

*Observed dehydro. values divided by 0.7 to correct for incomplete separation as measured on known solutions.

†Gamma of dehydro. converted to I.U. (100 gamma of androsterone) on basis of activity ratios by the inunction assay method. 100 gamma of androsterone equal to 320 gamma of dehydro.

‡A. equals androsterone.

§D. equals dehydroisoandrosterone.

digitonide was decomposed with pyridine and the dehydroisoandrosterone and androsterone fractions assayed in parallel with the pure androgens. An extract of normal male urine was treated similarly. Control experiments with known solutions of dehydroisoandrosterone gave only a 70% recovery with digitonin treatment so the values observed for the urine fractions have been corrected for this loss. The results are shown in Table I.

The value found for the total androgen excretion of male castrates, 6 I.U. per day, agrees with the values previously reported, most of which are between 1 and 10 I.U. per day although Callow reports one case with 30-39 I.U. per day. The ratio of androsterone to dehydroisoandrosterone in male castrate urine, 4:1, contrasts with the approximately 1:1 ratio previously reported for normal male urine[7, 8] and the 3:2 ratio found here. Attempts to isolate androgens from these fractions were unsuccessful. In the absence of actual identification of androsterone or dehydroisoandrosterone considerable caution must be used in interpreting these figures. On the other hand, in view of the presence of these two androgens in the urine of normal men and women and female castrates[7, 9, 10] it seems reasonable to assume that these two compounds make up the greater part of the biologically active material. (An inactive epimer of androsterone, alpha-3-hydroxyetiocholanone-17, has been isolated in amounts equal to androsterone from the urine of normal men and of female castrates.[7, 10]) Hansen[4] suggests that the androgenic material in male castrate urine resembles Δ-4-androstenedione in its biological action. He did not consider the possible presence of dehydroisoandrosterone and the properties he mentions can probably be explained as due to the combined actions of dehydroisoandrosterone and androsterone rather than to androstenedione.

It is apparent from Table I that the sum of the androgenic activities in the androsterone and dehydroisoandrosterone fractions is about 20% less than the total androgens as determined on the unfractionated urine. This difference is due to errors arising from the manipulations involved, the biological assay, the rather arbitrary correction factor applied to the dehydroisoandrosterone values and the activity ratio used in converting gamma of dehydroisoandrosterone to gamma of androsterone (I.U.). The existence of this difference and the nature of the methods used make it inadvisable to theorize from the present data but at the same time these ratios have interesting implications.

---

[8] Dingemanse, E., and Laqueur, E., *Biochem. J.*, 1938, **32**, 651.

[9] Callow, N. H., and Callow, R. K., *Biochem. J.*, 1939, **33**, 931.

[10] Hirschmann, H., *J. Biol. Chem.*, 1939, **130**, 421.

The isolation of steroids from the adrenal cortex closely resembling the common androgens, some having androgenic activity, and the high levels of urinary androgens in some cases of adrenal tumor seem to point to the adrenal as the source of the extragonadal urinary androgens. Callow[3] has discussed this possibility and in view of the isolation of large quantities of dehydroisoandrosterone from the urine of patients with adrenal tumors suggests that all the dehydroisoandrosterone and most of the other androgenic activity in normal male urine is derived from the adrenal and not the testis. With this suggestion in mind it is surprising to find that the male castrate urine contained a smaller rather than a larger proportion of dehydroisoandrosterone than normal urine. It is conceivable that the testis normally transforms a portion of the steroids elaborated by the adrenal cortex into the testis hormone which is subsequently changed by other tissues into suitable excretion forms, androsterone and its inactive epimer and dehydroisoandrosterone. A small part of the steroids appears to go through this or a similar series of reactions independently of the testis and can be found in the urine when the testis is absent as the same androgens but in slightly different proportions.

The measurement of the relative amounts of these two types of urinary androgens by digitonin fractionation is being carried out on the urine of female castrates, boys and girls, in an effort to extend the data.

Summary. A pooled urine collection from 2 male castrates contained 6 I.U. per day of androgenic activity. Capon assay of the results of digitonin fractionation indicated an androsterone to dehydroisoandrosterone ratio of 4:1 compared with the approximately 1:1 ratio found in normal male urine. In the absence of the testis smaller amounts of the same or similar androgens are excreted in slightly different proportions.

11279

Quantitative Studies on Relationship Between Estrogen and Mammary Gland Carcinoma in Strain C3H Mice.

Edward L. Burns and John R. Schenken. (Introduced by Kenneth L. Burdon.)

From the Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, New Orleans.

The first experimental proof of a qualitative and quantitative relationship between the internal secretions of the ovary and carcinoma of the mammary gland in mice was presented by Leo Loeb[1, 2] and more recent investigations[3] have amply confirmed the qualitative phase of this relationship. The following experiments concern the quantitative aspects of the problem.

Materials and Methods. The data were obtained from 206 strain C3H mice* of which 122 were experimental and 84 were control animals. Their diet consisted of fresh Purina dog chow, to which lettuce was added at irregular intervals.

All the experimental animals were males and all were at least 5 months old when they died or were sacrificed. They were divided into 7 groups (Table I) and were injected with estradiol benzoate in a solution of sesame oil (Progynon-B) from the age of 2 weeks onward as follows:

Group I received 3000 rat units in 2 doses over a period of 3 days.

Groups II-VI inclusive received 100 rat units weekly for 4, 8, 12, 16, and 20 weeks respectively.

Group VII received 100 rat units weekly throughout the duration of life.

The control groups consisted of 38 untreated breeding male (Group VIII) and 46 untreated breeding female mice (Group IX),

---

[1] Lathrop, A. E. C., and Loeb, Leo, *J. Cancer Res.*, 1916, **1**, 1.

[2] Loeb, Leo, *J. M. Research*, 1919, **40**, 477.

[3] Cori, C. F., *J. Exp. Med.*, 1927, **45**, 983; Murray, W. S., *J. Cancer Res.*, 1928, **12**, 18; Lacassagne, A., *Compt. rend. Acad. d. sc.*, 1932, **195**, 630; Bonser, G. M., *J. Path. and Bact.*, 1935, **41**, 33; Burrows, H., *Am. J. Cancer*, 1935, **24**, 613; Cramer, W., and Horning, E. S., *Lancet*, 1936, **1**, 247; Suntzeff, V., Burns, E. L., Moskop, M., and Loeb, Leo, *Am. J. Cancer*, 1936, **27**, 229.

* The parent animals were obtained in 1938 from the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. In New Orleans the strain has been propagated only by brother to sister matings. A high percentage of spontaneous carcinoma of the mammary gland occurs among the breeding females of this strain.

TABLE I.

Group	Experimental mice							Control mice	
	I	II	III	IV	V	VI	VII	VIII	IX
Incidence of Tumors in Relation to Dosage of Estrogen.									
No. of animals	20	11	20	14	19	19	19	38	46
No. of tumors	0	0	3	1	11	9	10	0	32
Incidence tumors (%)	0	0	15.0	7.1	57.8	47.3	52.6	0	69.5
Total dosage rat units	3000	400	800	1200	1600	2000	Avg 3270	0	0
No. of doses	2	4	8	12	16	20	Avg 32.7	0	0
Mode of dosage	Alternate days at age 2 weeks	weekly	weekly	weekly	weekly	weekly	weekly	0	0
Incidence of Tumors in Relation to Age in Months.									
Age range	6.30-19.83	8.86-18.80	5.13-19.46	6.20-18.30	6.00-13.36	5.43-15.53	5.66-12.46	6.00-18.66	6.93-18.86
Avg age at which injections were ended	0.53	1.06	2.10	3.00	4.04	4.94	7.90	—	—
Avg interval between last injection and death									
With tumors	—	—	4.83	3.10	5.93	4.20	0	—	—
Without tumors	13.50	10.00	7.73	7.76	3.10	5.53	0	—	—
Entire group	13.50	10.00	7.30	7.33	4.76	4.86	0	—	—
Avg age at death									
With tumors	—	—	6.90	6.60	9.86	9.06	8.60	—	12.96
Without tumors	14.03	11.06	9.83	10.60	7.40	11.56	7.06	14.03	9.86
Entire group	14.03	11.06	9.40	10.33	8.80	9.80	7.90	14.03	12.03

all of which were over 6 months of age at the time of necropsy (Table I).

It is important to note that after the specified course of injections the mice in Groups I-VI inclusive received no further treatment.

The presence or absence of tumors was determined by gross inspection at necropsy, a sufficient number of tumors having been examined microscopically to establish the accuracy of gross diagnosis. Small masses of questionable identity were not regarded as neoplasms.

Results. No tumors developed in the animals of Group I or Group II. A low incidence was observed in the animals of Groups III and IV, and the tumors developed at an earlier age in the mice of these groups than in the mice of any other experimental group. Moreover, all 3 animals which developed tumors in Group III were from the same litter. A relatively high and essentially equal percentage of tumors developed in mice of Groups V, VI and VII and the neoplasms occurred at approximately the same average age in each group. The female controls (Group IX) developed a higher percentage of tumors than any of the experimental groups, and the tumors appeared at an older age. No tumors were noted in the male controls (Group VIII). The complete data are shown in Table I.

Summary. 1. Beginning at the age of 2 weeks, 122 non-breeding male strain C3H mice were treated with estrogen for varying periods of time. At the end of the specified course of injections the mice received no further treatment until they died or were sacrificed. 2. Four weekly doses of 100 rat units of estrogen produced no mammary gland carcinomas; 8 and 12 weekly doses of 100 rat units produced a low incidence of these tumors. 3. After 16 weekly doses of 100 rat units of estrogen had been given a relatively high percentage of carcinoma of the mammary gland developed; the administration of 20 weekly doses or an average of 32.7 weekly doses of 100 rat units did not raise the incidence of tumors above this level. 4. Three thousand rat units of estrogen administered over a period of 3 days at the age of 2 weeks produced no tumors.

11280

Effect of Inhaled Carbon Dioxide in Rheumatoid (Atrophic) Arthritis.

Arthur Locke and Morris A. Cohen.

From the Institute of Pathology and the Department of Orthopedics of the Western Pennsylvania Hospital, Pittsburgh.

A part of the limitation to movement in rheumatoid (atrophic) arthritis is due to muscle spasm and pain, not to limiting changes in structure. It can be lessened through administration of carbon dioxide and returned through acceleration of carbon dioxide loss (over-breathing) to a degree suggesting relationship to the phenomenon of hyperventilation tetany.

The 10 subjects of the experiment summarized in Table I were given inhalations of 10 to 15% carbon dioxide in oxygen for 2 to 5 minutes, from a Tissot spirometer, following determination of the degree of limitation to motion existing prior to inhalation. All responded to the inhalation with the immediate, transient, measurable and significant decrease in limitation indicated. A subjective impression of decrease in pain was reported, following first inhalation of CO_2, by all of the group but one. It could be corroborated by observation, before and after inhalation, of the amount of passive flexion or pressure required to elicit the painful sensation in question, in only 6. The effect on pain, like the effect on limitation of motion, was not observed following inhalation of oxygen unmixed with CO_2 but was more certainly elicited by mixtures of CO_2 with oxygen than by mixtures of CO_2 with air. Both effects persisted longest in those of the subjects making greatest effort to guard against over-breathing through self-control, stimulation of CO_2-production through persevering, mild, non-painful exercise, and precaution against chilling. Cumulative effect, following repeated inhalation, was obtained only up to the point of limitation by factors other than muscle spasm and pain.

Carbon dioxide was less certain and definite in effect on the conditions grouped in Table II.

Administration of 10 to 20% CO_2 in oxygen to rabbits was followed by the transient decrease in responsiveness to the stimulus of chilling[1] indicated in Fig. 1 and the associated[2] transient decrease in

---

[1] Locke, A., *J. Immunol.*, 1939, **36**, 159. 365.

[2] Locke, A., and Main, E. R., *J. Immunol.*, 1939, **36**, 173.

TABLE I.

Transient Decrease in Limitation to Movement Imposed by Muscle Spasm and Pain, in Rheumatoid (Atrophic) Arthritis, Following Inhalation of 10 to 15% CO_2.

Subject	No. of consecutive daily inhalations given	Extent of freedom permitted in the movement described			Indication of a decrease in pain as appraised by		Hr. duration of the combined effect
			Before CO_2 inhalation	After	Subject,	Observer	
DB	1	Active flexion, knee	80°	complete	+	+	10
BB	1	” ” knees	0°	0°			
	8			15,20°			
	14			15,20°			
	1	Upward motion, from bed: shoulders, elbows, heels	0 in.	3;1,6;0 in.	+	+	6-12
	8			4;6,10;4 in.			>12
	14			6;7,10;5,7 in.			retained
MC	1	Active flexion, left elbow	5°	10°			
	6			30°			
	34			40°			
	1	” ” right knee	10°	10°	+	+	2
	16			20°			6-12
	34			35°			retained
	1	Raising right arm	110°	170°			
	1	Separation, teeth	¼ in.	¼ in.	(unable to eat solid food)		
	6			½ in.	(eating solid food)		
EW	1	Passive flexion, knees	40,50°	50,60°	+		6-12
LD	1	” ” left elbow	0°	8°	+	+	1- 2
JP	1	Clenching hand	partial	complete	+		8
HG	1	Walking	limps	still limps	+		
	8		walking normally		+		
DN	1	Motility of arms and shoulders increased			+	+	6
VW	1	Motility of fingers increased			+	+	6-12
RF	1	” ” ” ”					

TABLE II.

A Related but Less Frequently Elicited, Less Definite and Less Specific Effect in Conditions with Joint Stiffness, Pain or Incapacitation not Caused by Rheumatoid (Atrophic) Arthritis.

Subject	No. of consecutive daily inhalations given	Extent of freedom permitted in the movement described:			Indication of a decrease in pain as appraised by		Hr. duration of the combined effect
			Before CO_2 inhalation	After	Subject,	Observer	
Osteo-arthritis:							
CB	1	Sitting up	to 60°	to 90°	+	+	6-12
NC	1	Passive flexion, knees	120,125°	130,137°			
PM	1	No measurable change			+		1
AR	1	” ” ”			+		6-12
Fibrositis:							
EM	1	” ” ”			+	+	1
EC	1	” ” ”					
OL	L	” ” ”			+		<1
Arthroplasty, post-operative:							
CA	1	Active flexion, elbow	0°	15°	+		7
E. coli Infection:							
CT	1	Motility of legs increased			+	+	3-4
Charcot Knee:							
MB	1	Ascending stairs	0	0	+	+	3-4
	7			+			>12

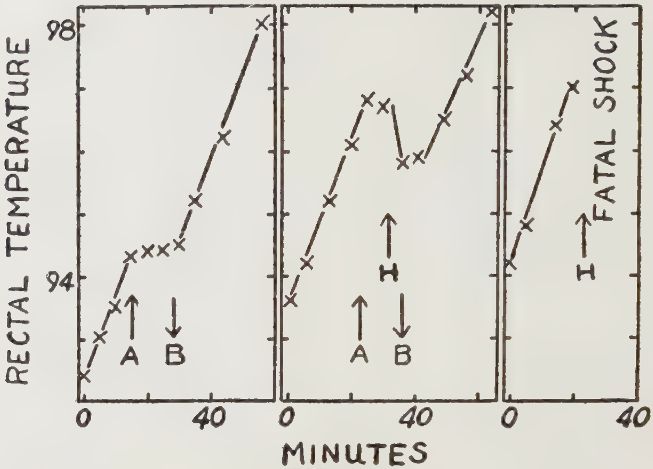


FIG. 1.

Effect of inhaled 10% CO_2 (in O_2) on responsiveness to stimulus and on susceptibility to histamine shock in chilled rabbits. The crosses indicate the course of spontaneous temperature recovery following chilling to the beginning temperatures indicated. A marks the time at which inhalation was begun; B marks the time of discontinuance; 0.8 mg of histamine was given, intravenously, at the time marked H.

susceptibility to histamine. Carbon dioxide raises the threshold of sensitivity to stimulus[3] to an extent capable of producing anesthesia[4] on inhalation for 1 minute or longer in concentrations exceeding 20%. Histamine is the possible chemical mediator of pain.[5]

Mixtures of carbon dioxide with air or oxygen have been given over long periods of time in amounts and concentrations in excess of those given the subjects in Tables I and II, without ill effect and with many indications of beneficial effect (improvement in peripheral circulation).[6, 7] Marked relaxation was observed by Loevenhart,[8] during administration of CO_2 to a group of psychotics for the purpose of rousing them from catatonia. Spontaneous movements of the extremities occurred. The eyes lost their fixed, staring quality and undertook purposeful movement. Carbon dioxide was given by Henderson[6] for the purpose of obtaining increase in collateral circulation in angina pectoris and intermittent claudication. Unmistakable increase in exercise tolerance was obtained. Kerr[9] and associates have used CO_2 to combat anxiety complex, effort syndrome and related conditions precipitated by hyperventilation and characterized by impaired ability to relax and to utilize relaxation as a means of conserving and accumulating strength. Over-breathing is a constant hazard when pain[10] is combined with inactivity. Maintenance of an adequate circulation is the major defense not only against low-grade infection[1] but also against atrophy and the type of structural change associated with arthritis.[11]

---

[3] Hettwer, J. P., *Am. J. Physiol.*, 1938, **122**, 275.

[4] Leake, C. D., and Waters, R. M., *J. Pharm. and Exp. Therap.*, 1928, **33**, 280.

[5] Rosenthal, S. R., and Minard, D., *J. Exp. Med.*, 1939, **70**, 415.

[6] Henderson, Y., *Am. Heart J.*, 1930-31, **6**, 548.

[7] Dautrebande, L., *et al.*, *Ann. physiol. physicochim. biol.*, 1938, **14**, 516; Klingman, T., *Ann. Int. Med.*, 1939, **13**, 677.

[8] Loevenhart, A. S., Lorenz, W. F., and Waters, R. M., *J. Am. Med. Assn.*, 1929, **92**, 880.

The observations reported above suggest, but do not establish, the possibility that CO_2-impoverishment may be a factor in the development and progress of atrophic arthritis. Work is under way on the larger problem of therapy. No conclusive progress in that direction has been obtained or is herewith implied.

Summary. A transient, small but measurable and significant decrease in limitation to motion was observed in 10 persons with atrophic arthritis following inhalation of 10 to 15% CO_2 (in O_2) for a length of time (2 to 5 min) sufficient to induce a feeling of warmth and breathlessness. An equivalently definite and regularly elicited effect was not observed in 10 additional persons with limitations due to conditions (osteo-arthritis, fibrositis, etc.) not classifiable as atrophic arthritis. Transient decrease in sensitivity to cold and in susceptibility to histamine was observed in rabbits during inhalation of CO_2 in O_2.

---

[9] Kerr, W. J., Dalton, J. W., and Gliebe, P. A., *Ann. Int. Med.*, 1937, **11**, 961; Soley, M. H., and Shock, N. W., *Am. J. Med. Sci.*, 1938, **196**, 840.

[10] Henderson, Y., *Am. J. Physiol.*, 1909-10, **25**, 310.

[11] Goldhaft, A. D., Wright, L. M., and Pemberton, R., *Am. J. Med. Sci.*, 1930, **180**, 386.

11281

Intravenous Administration of Bovine and Human Plasma to Man: Proof of Utilization.*

OWEN H. WANGENSTEEN, HARRY HALL, ARNOLD KREMEN AND BEATRICE STEVENS.

From the Department of Surgery, University of Minnesota Medical School, Minneapolis, Minn.

In this and other clinics, human plasma has been given intravenously in preparing patients with obstruction at the gastric outlet, exhibiting the effects of hypoproteinemia, for operation. Whipple[1, 2] and his associates have demonstrated that dog plasma administered intravenously to dogs is utilized. In this study it is proposed to indicate that: (1) Human plasma administered intravenously to patients is retained and utilized, (2) plasma presents distinct advantages over whole blood for the purpose of maintaining nitrogen equilibrium and to elevate depleted plasma proteins in starvation states, (3) bovine plasma may be given intravenously to man in fairly large quantities; it too is retained and utilized.

We wish to make it clear that the intravenous administration of bovine plasma to man has not been established as a safe routine hospital procedure. The data submitted herewith afford proof, however, that bovine plasma may be administered intravenously to some patients in fairly large quantities. When the possibilities and limitations of the method become understood well enough, it is not unlikely that the method may become a practical hospital procedure useful in civil as well as in war surgery for various purposes having to do with contracted blood volumes and protein stores.

Method. All the patients upon whom metabolic tests were made in this study and to whom human and later bovine plasma was given, were afflicted with cancer and presented some of the effects of protein starvation. All patients, however, were essentially afebrile. The human plasma was obtained from the surgical blood bank of citrated blood in the hospital, the cells being removed by centrifuga-

---

* The researches presented herewith were supported by grants of the Graduate School of the University of Minnesota and by a grant for technical assistance by the Work Projects Administration, Official Project No. 665-71-3-69, Sub-project No. 258.

[1] Pommerenke, W. T., Slavin, H. B., Kariher, D. H., and Whipple, J. G., *J. Exp. Med.*, 1935, **61**, 283.

[2] Daft, F. S., Robscheit-Robbins, F. S., and Whipple, J. G., *J. Biol. Chem.*, 1938, **123**, 87.

tion. The bovine plasma was prepared similarly, (10 cc of a 2.5% sodium citrate solution per 100 cc of blood) the blood, after collection from a living animal by venipuncture, being placed in centrifuge tubes with a capacity of 250 cc. Centrifugation was carried out for one hour at 2500 revolutions per minute. The supernatant plasma was pipetted off into sterile flasks for storage in the ice box (temperature 5°C) where it was allowed to remain for a week or more before being used. The sterility of the bovine plasma was determined by culture on veal infusion broth before administration. Several batches of plasma were passed through a Berkefeld filter as an added precaution, but extensive trial showed that this procedure was not necessary to insure sterility. Sulfanilamide, 0.2 gram to a liter of plasma, was used in some instances as a preservative (Novak).[3] Through the coöperation of the Department of Veterinary Medicine† of the University of Minnesota, it was possible to secure fairly large weekly stores of bovine blood from cows that were free from tuberculosis and Bang's disease.

During the period of the study, the patients were on a protein-free diet. Tea, orange juice, nectar and water were allowed by mouth to supplement intravenous administration of 5 or 10% glucose solution. Enough carbohydrate was given in this manner to afford a caloric intake of about 1200 calories daily and to avoid ketosis.

The total daily output of urine passed was preserved with toluene. Daily determinations of the total urinary nitrogen,[4] non-protein nitrogen, urea[5] nitrogen and sugar were made. At the beginning of the study and at the end, and at intervals of 2 to 3 days between, values for plasma proteins, non-protein nitrogen[5] and blood urea nitrogen[6] and chlorides[7] were determined. The specific gravity of the blood of the recipients was kindly determined for us by Dr. C. J. Bellis by the falling drop method.[8] The weight of the patient was determined at intervals through the period of study.

As indicated in Table I, the usual procedure was to allow the excretion of nitrogen in the urine to reach a basal level, the patient being supported by a carbohydrate intake only in the 3- to 5-day

---

[3] Novak, M., *J. A. M. A.*, 1939, **113**, 2227.

† It is a real pleasure to acknowledge here our gratitude for helpful assistance to the late Dr. C. P. Fitch and to Dr. Willard L. Boyd, Professors of Veterinary Medicine.

[4] Koch, F. C., and McMeekin, T. L., *J. Amer. Chem. Soc.*, 1924, **46**, 2066.

[5] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81, 111.

[6] Karr, W. G., *J. Lab. and Clin. Med.*, 1924, **9**, 329.

[7] Cavett, J. W., and Holdridge, C. E., *J. Lab. and Clin. Med.*, 1933, **18**, 944.

[8] Barbour, H. F., and Hamilton, W. F., *J. A. M. A.*, 1927, **88**, 91.

TABLE I.

Metabolic studies attending intravenous administration of human blood and plasma and bovine plasma

		Urine				Blood							
						Plasma Protein							
	Period 1939	Total N_2 g	N.P.N. g	Urea N_2 g	Sugar g	Total	Albumin	Globulin	N.P.N. mg%	Urea N_2 mg%	Spec. Grav.	Protein Intake cc	Weight lb
Case No. 1:	5/10- 5/13	20.64				6.8	3.7	2.8	40.6		1.057	0	
	Av. Daily	5.16											
	5/14- 5/18	19.34				6.8	4.2	2.7			1.063	2250 H.Pl. I-v.	118½
	Av. Daily	3.87											
	5/19- 5/24	29.904	29.314	17.863		8.8	5.1	3.8			1.063	3000 H.Bl. I-v.	115
	*Av. Daily	5.981	5.863	3.573									
Case No. 3:	7/ 1- 7/ 3	21.708	20.146	11.087	262.71	6.1	2.9	3.2	27.1	8.2	1.045	0	143
	Av. Daily	7.236	6.715	3.696	87.57								
	7/ 4- 7/ 6	22.833	22.123	13.268	69.388	5.8	2.6	3.0		10.7	1.052	1500 H.Bl. I-v.	138
	Av. Daily	7.611	7.374	4.423	23.128								
	7/ 7- 7/10	17.360	17.793	11.236	367.064	6.9	3.2	3.4	41.1	9.7	1.050	1000 B.Pl. I-v.	141
	Av. Daily	4.340	4.444	2.809	91.766								
Case No. 7:	10/ 4-10/ 8	38.332	33.826	22.982	96.38	7.48	4.14	3.34	26.4	17.5		0	126
	Av. Daily	7.666	6.766	4.596	19.27								
	10/ 9-10/12	21.410	25.629	12.173	83.475	7.18	5.00	1.52	41.4	9.61		2000 H.Bl. I-v.	130
	Av. Daily	5.353	6.407	3.044	20.869								
	10/13-10/17	29.168	30.084	19.283	103.546	7.14	4.3	2.84	48.0	20.4		1500 B.Pl. I-v.	133½
	Av. Daily	5.834	6.107	3.857	20.709								

* Part of one daily specimen lost. This day omitted from calculation of total and average daily N_2 Excretion.

Case 1. Mr. F. K., Age 56, U. H. No. 680697 (Carcinoma of esophagus; Gastrostomy 5/8/39).
Case 3. Mr. W. M., " 53, U. H. No. 680255 (" " stomach; Exploratory Laparotomy 6/27/39).
Case 7. Mr. H. W., " 64, U. H. No. 684341 (" " lower esophagus and stomach; Jejunostomy 9/19/39).

interval. The effect of the administration of human blood or plasma upon the excretion of nitrogen was noted first; then, similar studies were made during the administration of bovine plasma. The daily administered amounts of human blood or plasma and bovine plasma indicated in the table were given in 2 divided doses (morning and evening). An intracutaneous injection of 0.05 cc of bovine plasma was first made as a test. If no enlargement of the wheal was observed at 20 and 40 minutes, the test was interpreted as being negative and 2 cc of bovine plasma was injected intravenously, before larger amounts were given. None of the patients were diabetic and the urinary excretion of sugar represents spillage from too rapid administration of 10% glucose solution.

Results. Representative results are indicated in the table. For purposes of conserving space, the detailed information in Cases 2, 4, 5 and 6 are omitted in the table. Only Cases 1, 5 and 6 failed to receive bovine plasma intravenously. Case 2 received 750 cc of bovine plasma over a 4-day interval; Case 4 received 1000 cc of bovine plasma and the daily urinary excretion of nitrogen was definitely lower than in the 4 days preceding during which 2000 cc of whole human blood was given. Tests for protein in the urine were uniformly negative; in all instances, the total urinary nitrogen was accounted for by non-protein nitrogen. The urinary nitrogen studies in Case 1 (see table) suggest a definitely greater urinary excretion of nitrogen during intravenous administration of whole human blood than when human plasma was given. This observation appears to be borne out in other instances, *viz.,* that administration of whole blood pyramids the urinary excretion of nitrogen. In Case 5, after 4 days of protein starvation, the urinary excretion of nitrogen fell to 1.26 g per day; 500 cc of whole human blood was then given intravenously for 6 days. On the 5th day the urinary excretion of nitrogen was 9.12 g, on the 6th day 8.3 g. In Case 1, over the 5-day period that 2250 plasma was given (protein content 7 g per 100 cc), a total of 157.5 g of protein or 25.3 g of nitrogen was introduced. During this same time 19.34 g of nitrogen was excreted in the urine, indicating a nitrogen retention of 6 g.

In Case 3 (see table), the average daily urinary output of nitrogen was 7.61 g after 3 days of daily administration of 500 cc of whole human blood intravenously, preceded in turn by 3 days of no exogenous intake of protein. Accompanying 4 days of administration of bovine plasma, during which time 800 cc had been given, the daily urinary nitrogen excretion was 4.34 g, suggesting that the intravenously administered protein was being stored somewhere in the body. Determination of the protein content of bovine plasma was made on 4 batches. Values between 7 and 8 g per 100 cc were uniformly found. Case 7 (see table) received a total of 1500 cc of bovine plasma over a 5-day period, without increasing the daily urinary excretion of nitrogen appreciably over what it had been after daily administrations of 500 cc of whole human blood over a 4-day period.

In no instance did we succeed in keeping the patient in nitrogen balance attending intravenous injection of bovine plasma. In Case 3, there was an excretion of 7.1 g more of nitrogen in the urine over the intake, during the 4-day period of the study. Similarly in Case 7, there was a negative nitrogen balance of 9.968 g for the 5-day period. However, in Case 3, the average daily excretion of nitrogen was definitely less than the basal level for the control period when there was no exogenous intake of nitrogen. While in Case 7, the daily average excretion of nitrogen in the urine, during the period when bovine plasma was given, was less than the basal level of the preliminary 4-day period of protein starvation. Further, few significant increases in the non-protein nitrogen of the blood occurred during the administration of bovine plasma. The relationship between the total non-protein nitrogen and the urea of both blood and urine showed no significant alteration suggestive of abnormal catabolism of protein.

Inasmuch as Case 3 was given only an average of 2.5 g of nitrogen per day and Case 7, 3.84 g, it is reasonable to infer that not enough protein (bovine plasma) was given to maintain a positive nitrogen balance. In Case 1, a positive nitrogen balance was maintained with human plasma, an average of 5.06 g of nitrogen being given each day of the 5-day period of study. Inasmuch as enough bovine plasma was not given to maintain nitrogen equilibrium, one could not reasonably expect that the plasma proteins of these patients would be increased.

To a patient with a bleeding gastric ulcer upon whom no metabolic studies were made, 25 and 100 cc of bovine plasma were injected intravenously on 2 successive days without reaction. Two days later when bleeding to a shock level occurred (blood pressure 80/40), 300 cc of bovine plasma was given over an hour's time; during the next 2.5 hours an additional 400 cc was given—all without reaction, the blood pressure having risen after the initial injection of 300 cc to 130/90, at which level it was maintained. This was the largest amount of bovine plasma injected over a relatively short period of time.

Reactions. Bovine plasma has been given intravenously in small amounts (2 to 10 cc) to a larger number of patients who will be reported upon in a later study. It may be said here, that of 66 patients so injected, reactions were noted in 10 cases (15%). Nine of these reactions were classified as mild to moderate in severity. In a patient with an inoperable carcinoma of the rectum, having a history of asthma dating back 20 years, a severe anaphylactoid type of reaction with severe dyspnoea attended the intravenous injection of 2 cc of bovine plasma. The patient responded satisfactorily to intravenous injections of epinephrin and aminophylin. The patient had no late effects.

A thermal reaction was noted in Case 3. In 3 of the patients given large quantities of bovine plasma upon whom metabolic studies were made, a generalized urticaria developed 5 to 6 days after the administration of bovine plasma was begun. The urticaria lasted usually 3 to 4 days and was fairly satisfactorily relieved by epinephrine. There was no joint manifestations.

Three patients in the series were given a single intravenous injection of bovine plasma in the amounts of 100, 50 and 25 cc after 8, 7 and 48 days respectively had elapsed since the last injection. No reactions attended these injections.

Discussion. It would appear that the use of bovine plasma for the treatment of clinical states, in which contracted blood volumes or decreased protein stores are present, may have real promise. Before intravenous use of bovine plasma can be recommended for clinical usage, however, it is important to determine with some precision what the limitations of the method are with reference to safety of administration. It may prove that partition of the proteins in the plasma, *viz.*, administration of the albumen or the globulin fraction alone may prove more useful than the whole plasma.

Conclusions. 1. Human plasma administered intravenously is retained and utilized. A patient to whom a daily average of 450 cc of plasma was given over a 5-day period was maintained in positive nitrogen balance. 2. Human plasma appears to be a better agent than blood to employ to maintain nitrogen equilibrium in starvation states. 3. Bovine plasma can be given intravenously to man in fairly large quantities; it is retained and apparently utilized.

11282 P

N^1-Dodecanoylsulfanilamide. I. Experimental Infections with Beta Hemolytic Streptococci.

DAVID R. CLIMENKO AND R. L. SCHMIDT. (Introduced by Harry Gold.)

From the Pharmacological Laboratory, The Calco Chemical Company, Cold Spring Harbor

The chemical properties of N^1-dodecanoylsulfanilamide have been described by Crossley, Northey and Hultquist[1] and the absorption, distribution, and toxicity of the drug will be discussed in another paper of this series. The present report deals with the chemotherapeutic effects of the drug in experimental infections of mice with beta hemolytic streptococci.

While 3 strains of beta hemolytic streptococci, C-203,* SH-1685,† and "Todd",‡ have been employed in this work, the results have been so consistent that the report will only deal with the effects on C-203. Virulence titrations of a 16-hour culture of this organism grown on fresh beef infusion, whole blood media give 100% mortality figures within 24 hours for concentrations down to 1×10^{-6} cc and 100% mortality figures within 72 hours for concentrations down to 1×10^{-7} cc. These virulence figures refer to young mice of the Swiss strain weighing between 10-12 g. The absorption of N^1-dodecanoylsulfanilamide from the gastrointestinal tract seems to be dependent on the absorbable lipoids present in the gut. This fact manifested itself to us in our earlier experimental work when it appeared that the drug had no protective action in streptococcal infections after it had been administered either orally or subcutaneously in acacia suspension. The following experiment illustrates this point.

Experiment 1. All animals infected by the intraperitoneal administration of 1×10^{-6} cc of a 16-hour culture of C-203.

Group A. 10 mice. Untreated controls.

Group B. 10 mice. Sulfanilamide controls. 1 cc of a 1% acacia suspension of sulfanilamide administered orally 1 hour and 24 hours after infection.

Group C. 10 mice. Experimental series. One cc of a 1% acacia suspension of N^1-dodecanoylsulfanilamide administered subcutaneously 1 hour and 24 hours after infection.

---

[1] Crossley, M. L., Northey, E. H., and Hultquist, M. E., *J.A.C.S.*, 1939, **61**, 2950.

* From the Johns Hopkins Hospital, Baltimore, Md.

† From the National Institute of Health, Washington, D. C.

‡ From the Lilly Laboratories, Indianapolis, Ind.

TABLE I.

Group	% Mortality at 12 hr	24 hr	48 hr	72 hr	96 hr
A	20	100	—	—	—
B	0	0	10	20	20
C	20	100	—	—	—
D	10	100	—	—	—

Group D. 10 mice. Experimental series. One cc of a 1% acacia suspension of N^1-dodecanoylsulfanilamide administered orally 1 hour and 24 hours after infection.

The evidence elicited from the study of the absorption and distribution of the drug suggested that it would be necessary to administer the drug together with fats capable of being absorbed from the gastrointestinal tract if any therapeutic effect was to be demonstrated. Accordingly all subsequent experiments involved the administration of the drug orally in olive oil or milk and oil suspensions. When administered in this manner, the drug appeared to be somewhat more effective against beta hemolytic streptococcal infections than sulfanilamide itself.

Experiment 2. All animals infected by the intraperitoneal administration of 1 x 10^{-6} cc of a 16-hour culture of C-203.

Group A. 100 mice. Untreated controls.

Group B. 250 mice. Sulfanilamide controls. One cc of a 1% acacia suspension administered orally 1 hour and 24 hours after infection.

Group C. 200 mice. Experimental series. One cc of a 1% olive oil solution of N^1-dodecanoylsulfanilamide administered orally 1 hour and 24 hours after infection.

Group D. 100 mice. Experimental series. One cc of a 1% suspension of N^1-dodecanoylsulfanilamide in milk and oil administered orally 1 hour and 24 hours after infection.

As is the case with sulfanilamide itself, N^1-dodecanoylsulfanilamide shows no protective action against overwhelming infections.

Discussion. An exhaustive pharmacological study of this drug is being carried out at the present time and the results will be reported in

TABLE II.

Group	% Mortality at 12 hr	24 hr	48 hr	72 hr	96 hr
A	26	100	—	—	—
B	0	2	8	10	16
C	0	0	2	4	4
D	0	1	1	1	1

the immediate future. It has been suggested[2] that the activity of the drug is dependent on its breakdown with a resultant liberation of sulfanilamide. Our present direct evidence shows that no such breakdown occurs in the gastrointestinal tract. Indirect evidence from studies on therapeutic efficacy[3] indicates that this N^1-acyl compound is more active than sulfanilamide on a molecular basis.

Results. 1. N^1-dodecanoylsulfanilamide, when administered in oil to mice, shows marked therapeutic efficacy against beta hemolytic streptococci. 2. This therapeutic effect is lost when the drug is administered in an aqueous medium.

11283 P

N^1-Dodecanoylsulfanilamide. II. Experimental Infections with *Mycobacterium tuberculosis.*

David R. Climenko. (Introduced by Harry Gold.)

From the Pharmacological Laboratory, The Calco Chemical Company, Cold Spring Harbor

It was pointed out[1] that N^1-dodecanoylsulfanilamide, when administered in fatty menstrua, exerted a more potent therapeutic effect against experimental beta-hemolytic streptococcal infections than sulfanilamide. This compound, whose chemical and physical properties have been described,[2] differs from sulfanilamide only in so far as a long chain fatty acid has been substituted for an H atom in the N^1 position. This gives to the compound a very high degree of fat solubility.

A new impetus has been lent to the subject of the chemotherapy of tuberculous infections by the report of Rich and Follis[3] that sulfanilamide is capable of inhibiting the development of the tuberculous process in guinea pigs after the experimental infection with human tubercle bacilli. Greey and his associates[4, 5] have repeated these observations, but Kolmer, Raiziss and Rule[6] and Smithburn[7] have been unable to reproduce these results. Smithburn produced an experimental tuberculous meningitis, and administered the drug intraperitoneally, while Kolmer and his associates administered the drug intramuscularly. We have always found that oral administration of sulfanilamide is the most effective method of administration.

---

[2] Marshall, Jr., E. K. Personal communication.

[3] Feinstone, W. H., Wolff, R. and Williams, R. D. To be published.

[1] Climenko, D. R., and Schmidt, R. L., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 622.

[2] Crossley, M. L., Northey, E. H., and Hultquist, M. E., *J.A.C.S.*, 1939, **61**, 2950.

[3] Rich, A. R. and Follis, A. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 77.

[4] Greey, P. H., Campbell, H. H., and Colly, A. W., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 22.

[5] Greey, P. H., Boddington, G. D. H., and Little, M. H., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 448.

The fatty acids of chaulmoogra oil have been suggested for the treatment of tuberculous infections for some time. Walker and Sweeney[8] made this suggestion in 1920, while Rogers[9] and Burgess[10] reported clinical successes in the treatment of the dermal manifestations of tuberculous infections with hydnocarpic acid esters. Walker and Sweeney pointed out that chaulmoogric acid was not capable of penetrating the wall of the tubercle, and Kolmer, Davis and Jager[11] demonstrated that chaulmoogric acid had no inhibiting effect on the development of experimental tuberculous infections in guinea pigs.

The combination of a long chain fatty acid with sulfanilamide, combining the fat solubility of the one with the penetrating properties of the other, might provide a more effective agent in controlling experimental tubercular infections in guinea pigs than was available in sulfanilamide itself or in any member of the fatty acid series itself. It was with this specific purpose in mind that N¹-dodecanoylsulfanilamide was synthesized.

It was first demonstrated that N¹-dodecanoylsulfanilamide was capable of inhibiting the growth of human tubercle bacilli *in vitro*. A series of flasks containing beef infusion, glycerine, dextrose broth were set up to contain N¹-dodecanoylsulfanilamide in concentrations of 10 mg/100 cc, 20 mg/100 cc, and 100 mg/100 cc. A similar preparation was made containing sulfanilamide at a concentration of 100 mg/100 cc. A series of untreated control flasks were also set up. All flasks were inoculated with the H.37 strain of human tubercle bacilli and incubated at a temperature of 37.5°C ± 2°C for a period of 90 days. Concentrations of 10 mg/100 cc of N¹-dodecanoylsulfanilamide in the culture medium inhibited growth over this period, while a similar inhibition was produced by a concentration of 100 mg/100 cc of sulfanilamide. The controls grew out luxuriously.

---

[6] Kolmer, J. A., Raiziss, G. W., and Rule, A. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 581.

[7] Smithburn, K. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 574.

[8] Walker, E. L. and Sweeney, M. A., *J. Infect. Dis.*, 1920, **26**, 238.

[9] Rogers, L., *Brit. Med. J.*, 1933, **1**, 47.

[10] Burgess, W., *Brit. Med. J.*, 1935, **2**, 835.

[11] Kolmer, J. A., Davis, L. C. and Jager, R., *J. Infect. Dis.*, 1921, **28**, 265.

The *in vivo* work was carried out on guinea pigs. A group of 80 animals, weighing between 350-400 g were infected by the subcutaneous administration of 1 mg of the H.37 strain of human tubercle bacilli, administered in the region of the groin. These animals were then divided into 4 groups of 20 as follows: Group A. Untreated controls. Group B. Sulfanilamide series. 100 mg sulfanilamide in 1% acacia suspension administered daily by stomach tube for 45 days. Treatment initiated immediately after infection. Group C. Experimental series. 100 mg N^1-dodecanoylsulfanilamide in 2% olive oil solution administered daily by stomach tube for 45 days. Treatment initiated immediately after infection. Group D. Experimental series. 100 mg N^1-dodecanoylsulfanilamide in 2% olive oil solution administered daily by stomach tube for 40 days. Treatment initiated 5 days after infection.

All animals in Group A developed the classical signs of tuberculous infection with human strain organisms. Tubercles formed at the site of inoculation, and in most instances, discharged through a secondarily infected sinus. These animals developed generalized tuberculous peritonitis and lymphadenitis with gross splenomegaly and liver involvement. Pulmonary involvement was rarely seen. With the exception of those animals sacrificed 30 days after infection for postmortem examination, all members of this group died within 65 days.

Animals of Group B developed localized tubercular lesions at the site of injection, which rarely showed secondary infections. Animals sacrificed at the end of 30 days for comparison with untreated controls show very slight generalized disease. The splenomegaly in this series was not nearly as pronounced as in the case of the controls and the glandular involvement was considerably less extensive. The results were essentially similar to those described by Rich and Follis and by Greey and his associates.

Animals of Group C showed localized tubercular lesions at the site of infection, which in some instances spread by direct contact to involve a considerable area of tissue. One animal (No. 51 in the series) developed a tuberculous orchitis, but showed no signs of generalized tuberculous lymphadenitis or peritonitis. A large proportion of these animals developed secondary infections at the site of injection, sinuses formed, and heavy purulent material was discharged. In most instances the secondary organisms were staphylococci. Animals of this group sacrificed 120 days after infection showed no gross splenomegaly as compared with the untreated controls. In no instance was there macroscopic evidence of tuberculous peritonitis or tubercles in the liver or kidneys.

Animals of Group D showed localized tubercular lesions at the site of infection and involvement of the adjacent lymphatics. Practically all of these animals developed discharging purulent infections at the injection site. They showed no gross splenomegaly, liver involvement or kidney involvement. There was no macroscopic evidence of generalized tuberculous lymphadenitis; in no instance did the disease process extend beyond the periaortic glands at the bifurcation of the aorta.

Conclusions. 1. N^1-dodecanoylsulfanilamide inhibits the growth of tubercle bacilli *in vitro* at a concentration of 10 mg/100 cc in beef infusion-dextrose-glycerine media over a period of 90 days. 2. N^1-dodecanoylsulfanilamide inhibits the development of the tuberculous process in guinea pigs infected subcutaneously with a human strain of tubercle bacilli.

11284 P

Production of Pneumonia in Rats by Intravenous Injection of Pneumococci.*

ALICE H. KEMPF AND W. J. NUNGESTER.

From the Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.

Rake[1] demonstrated that pneumonia could be produced in mice when the pneumococci were introduced by the intravenous route. The important factors were the strain and dose of the organism, and the breed of mice. Twelve of 87 mice had macroscopic lesions; 6 of the 87 failed to show any microscopic lesions. The pathology of the various stages encountered was clearly described.

Prior to this work, with a few exceptions, intravenous injection of pneumococci has failed to give rise to pneumonia. Although it has long been postulated that the origin of the infection might be by way of the blood stream, there has been little evidence to support it.

Employing the Schwartzman phenomenon, Witebsky, Neter, and Ward[2] were able to localize pneumococci injected intravenously in dermal lesions of rabbits.

---

* These studies were aided by a grant from the Horace H. Rackham School of Graduate Studies.

[1] Rake, Geoffrey, *J. Exp. Med.* 1936, **63**, 191.

[2] Witebsky, Ernest and Neter, Erwin, PROC. SOC. BIOL. AND MED., 1938, **38**, 187.

TABLE I.
Type I Pneumonia Resulting from Intravenous Injection of Pneumococci

Sterile Mucin Intrabronchially* cc	Pneumococci Intravenously cc	No. of rats Inoculated	Dead	% of Animals with Macroscopic Consolidation†
0.1	10^{-1}	4	4	100
0.1	10^{-2}	21	14	62
0.1	10^{-4}	5	2	0
0.1	10^{-5}	3	1	33
0.1	10^{-7}	4	1	25
0.0	10^{-2}	5	4	0
0.1	0.0	5	0	0

* Animals which did not develop pneumonia were shown to have small atelectatic lesions, evidence of intrabronchial inoculation.

† From one-fourth to an entire lobe was involved.

In our experiments, rats were inoculated intrabronchially with 0.1 cc of sterile mucin according to a technic previously described;[3] immediately thereafter, the animals were injected intravenously (using the tail vein) with various amounts of culture. The data are presented in Table I.

In interpreting these results it must be remembered that these animals received only one inoculation of organisms, many of which were distributed throughout the body and removed by the fixed phagocytic cells. It is noteworthy that in at least one rat, the disease was produced with as small an inoculum as 10^{-7} cc of culture.

Although in the past it has been generally impossible to produce pneumonia in experimental animals by intravenous injection of pneumococci, this may be accomplished in rats following intrabronchial inoculation of sterile mucin. The presence of mucus material in the lung or some comparable condition is a necessary predisposing factor for the localization of the pneumococci.

---

[3] Nungester, W. J. and Jourdonais, L. F., *J. Infect. Dis.*, 1936, **59**, 258.

11285

Effect of Gelatin on Power of Women to Perform Maximal Anaerobic Work.*

F. A. HELLEBRANDT, ROZELL RORK AND ELIZABETH BROGDON.
(Introduced by W. J. Meek.)

From the Department of Physiology, University of Wisconsin Medical School.

Glycine is present in gelatin to the extent of 25%. Its creatinogenic action is the basis for its use in the treatment of myopathies and simple fatigability (Boothby,[1] Wilder,[2] Tripoli and Beard[3]). However, evidence is accumulating which suggests that although the anaerobic reactions of muscle biochemistry involve creatine, such reactions may be little concerned with the performance of exercise in the steady state (Sacks,[4] Flock, Ingle and Bollman,[5] Millikan[6]). It would, therefore, seem highly desirable and possibly even necessary to use maximal anaerobic work in quantitative studies designed to test the influence of gelatin on muscular fatigue which are based upon the creatine-fixing function of glycine. Ray, Johnson and Taylor[7] reported that the daily administration of 60 g of gelatin to each of 6 men for a period of weeks was associated invariably with an increase in work capacity. No appreciable effect was demonstrable when comparable doses of gelatin were given to 4 women included in the study. The rate of work which produced exhaustion within a few minutes, the magnitude of the difference in muscle power exhibited by the male and female subjects, and the sex variation in the response to gelatin were all sufficiently unusual to warrant repetition of the observations made on women.

Methods. The subjects of the investigation were 6 young adult women all of whom had had professional training in physical education and were accustomed to severe physical activity. The exercise was performed on an electrodynamic brake bicycle ergometer equipped with a graphic voltmeter to record rate of working and the speed of

---

* Supported in part by funds from the Wisconsin Alumni Research Foundation.

[1] Boothby, W. M., *Proc. Staff Meet. Mayo Clinic*, 1934, **9**, 593, 600.

[2] Wilder, R. M., *Proc. Staff Meet. Mayo Clinic*, 1934, **9**, 606.

[3] Tripoli, C. J., and Beard, H. H., *Southern Med. J.*, 1938, **31**, 662.

[4] Sacks, J., *Am. J. Physiol.*, 1938, **122**, 215.

[5] Flock, E. V., Ingle, D. J., and Bollman, J. L., *J. Biol. Chem.*, 1939, **129**, 99.

[6] Millikan, G. A., *Physiol. Rev.*, 1939, **19**, 503.

[7] Ray, G. B., Johnson, J. R., and Taylor, M. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 157.

pedalling (Kelso and Hellebrandt[8]). Load and speed were standardized for each subject. Duration of exercise was the independent variable. The subject rode to exhaustion, the end-point for exhaustion being inability to maintain the voltage which served as an indicator of the rate of working. Fig. 1 is a reproduction of a typical record, showing the rapidity with which the voltage rises as the speed of pedalling increases and the constancy with which it is

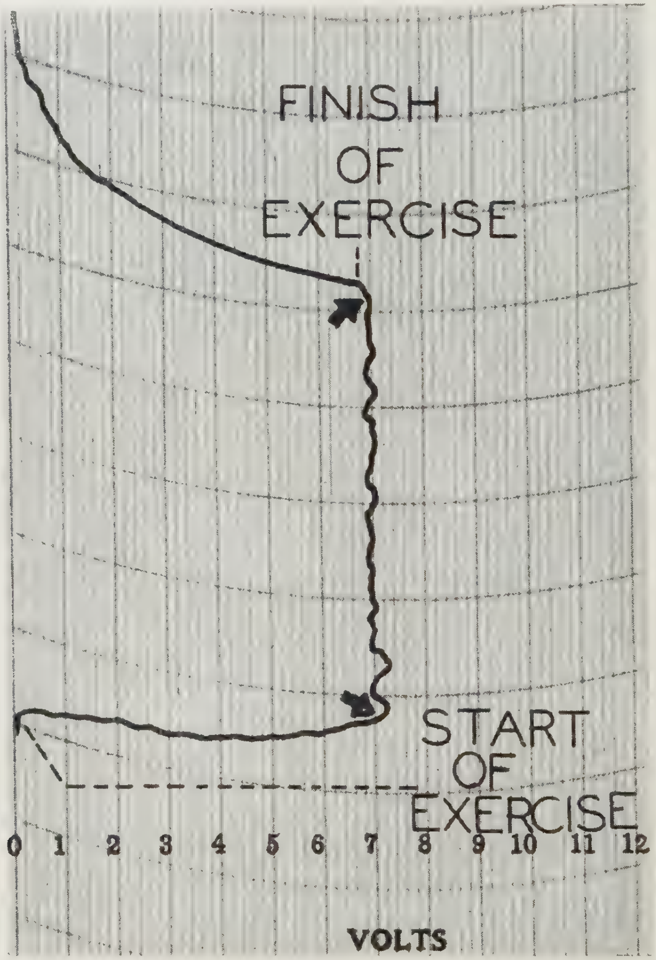


FIG. 1.
Typical graphic voltmeter record obtained on the electro-dynamic brake bicycle ergometer. Abscissae mark time in 10 seconds. Arrows indicate duration of exercise at the standardized rate of working.

---

[8] Kelso, L. E. A., and Hellebrandt, F. A., *J. Lab. and Clin. Med.*, 1934, **19**, 1105.

maintained once the rhythm is set. Activity ceased abruptly when the rpm necessary to sustain the voltage could no longer be tolerated. Five women worked at a 290 watt *subject output* and one at 220. Exhaustion was usually reached in less than 60 seconds. The duration of the effort in the record illustrated, beginning time at the instant the standard rate of working was achieved, was 45.4 seconds. These findings are in sharp contrast to the data reported by Ray, *et al.*, whose men and women subjects were rapidly fatigued by an *ergometer output* of 60 and 45 watts respectively.

After a period of preliminary training in the performance of rapid violent work standardized as to severity, gelatin was added to the regular diet and then withdrawn as exercise continued. Five subjects ingested 15 g of glycine *per diem*, administered as 60 g of sheet gelatin dissolved and then allowed to solidify in 250 cc of water to which the juice of one lemon and 2 tablespoons of sugar were added. One subject took half the dose in the form of granular gelatin dissolved in cold fruit juice.

Results and Their Interpretation. Fig. 2 illustrates the results obtained upon R. R., an especially well trained subject who had spent the summer preceding the experiment on a cycling tour. Her

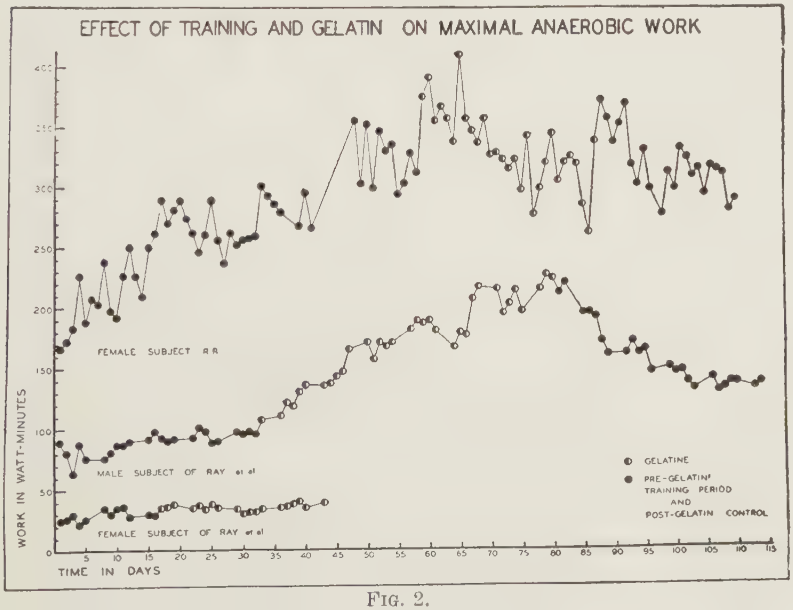


FIG. 2.

Subject R.R. exercised daily. Voltmeter records were not obtained on 8 days. In reproduction of the curves of Ray *et al.*,[7] it was necessary to assume an arbitrary 5 day week so that the number of observations published by them might correspond to the numerical value given for the duration of their experiment in days. This spreads their curve and minimizes the rate of change in power.

ability to perform anaerobic work of extreme severity increased over a period of 65 days. The daily ingestion of gelatin failed to sustain her improving capacity for work. The tendency toward a decrease in power, clearly evident in Fig. 2 (R.R.), may be attributed to "staleness." Though short-lived, the work was so rigorous that recovery may have been incomplete in 24 hours. No athlete can repeat a record-breaking performance daily. Transient increases in power occurred both at the beginning of the administration of gelatin and at the onset of the post-gelatin control period. No matter how well trained the subject, it is never possible to completely eliminate psychic influences in experimentation involving human beings performing work to exhaustion on devices not driven by forces beyond voluntary control.

For purposes of comparison, the only data reported for a woman by Ray, Johnson and Taylor[7] and those obtained on their male subject demonstrating the best effect attributed to gelatin are also reproduced in Fig. 2. Henderson and Haggard[9] estimated the maximal power of Yale Olympic oarsmen as 0.56 horse power in a short race lasting 6 minutes. The power developed by R. R. was 69% of that demonstrated by these men. This compares favorably with Dawson,[10] who estimates that the maximum energy which can be exerted by a woman is approximately 72% that of a man. The power developed by the male subjects of Ray, *et al.*, was 0.08 horse power, a rate of working which could be rapidly exhausting only if carried on at excessively high speeds. Subject R. R. rode at 98 rpm. In our experience, pedalling rates beyond 120 are self-limiting because of incoördination.

Fig. 3 illustrates the effect of introducing gelatin at different phases of the training period. Subject B. McL., slight in build and weighing 52 kilos, exhibited the greatest improvement with daily exercise and attained the highest work output in our series. The administration of gelatin relatively early in the experiment may be interpreted as having had no effect upon the trend of the training curve which rose steeply from the beginning to the end of the experiment. Her power to perform work oscillated more widely than that of R. E. B., whose training curve was closely approximated by our remaining subjects although gelatin was administered to them as early as to B. McL. The data presented in Fig. 3 suggest that gelatin neither affects the capacity to do more work as a result of repeated

---

[9] Henderson, Y., and Haggard, H. W., *Am. J. Physiol.*, 1925, **72**, 264.

[10] Dawson, P. M., *The Physiology of Physical Education*, 1935, Baltimore, The Williams & Wilkins Co.

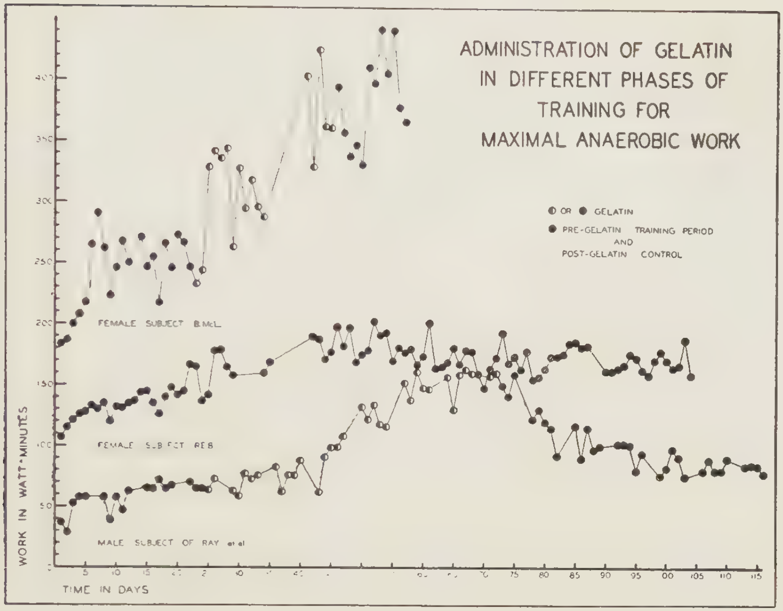


FIG. 3.

Voltmeter records were not obtained on days in which gaps occur in the curves of B. McL. and R.E.B. They exercised daily. In this figure as in Fig. 2, the 5-day week is arbitrarily used for graphing the data of Ray *et al.* against time.

activity, nor prevents the development of chronic fatigue (R. E. B.) when the activity is too severe.

The gelatin ingested was added to the regular diet, increasing its protein content by one gram or more per kilo *per diem.* Wishart[11] showed that the performance of a long distance racing cyclist was best on high protein diets. Mirski and his associates[12] have recently shown that the blood sugar and fuel reserves of the liver laid down on a protein-rich diet behave differently from those deposited on a diet of carbohydrate. Such observations suggest that gelatin might be advantageous in the performance of protracted hard work.

Summary and Conclusions. Physical exercise, maximal anaerobic in type and constant as to the rate of working and speed, was performed to the point of exhaustion by 6 healthy young adult women. After a period of preliminary training varying in length in the different subjects, gelatin was added to the diet and its influence upon the time of onset of muscular fatigue was noted. The evidence presented substantiates the following conclusions. Gelatin has no effect upon the capacity of women to perform maximal anaerobic work, nor any apparent effect upon the improvement in power due to training. Gelatin does not prevent the development of "staleness" when brief work of extreme severity is repeated daily over a long period of time. A comparison of the published data of Ray, *et al.,*[7] with that of our series of experiments suggests that the increase in work output attributed by these investigators to gelatin may also have been a training effect.

---

[11] Wishart, G. M., *J. Physiol.*, 1934, **82**, 189.

[12] Mirski, A., Rosenbaum, I., Stein, L., and Wertheimer, E., *J. Physiol.*, 1938, **92**, 48.

Acknowledgments. Our best thanks are due to Betty McLaughlin, Mary Stowell, Helene Sanger and Beatrice Hellebrandt for the faithfulness with which they "played themselves out" each time they rode the bicycle ergometer, and to Prof. Kelso of the School of Engineering for his analyses of the discrepancies in work evident in the comparison of our observations with those of Ray, Johnson and Taylor.

11286

Further Approach Toward Control of Spontaneous Cancer of Mammary Gland in Mice by Heptyl Aldehyde-Sodium Bisulphite.

LEONELL C. STRONG.* (Introduced by S. Bayne-Jones.)
From the Department of Anatomy, Yale University School of Medicine

Evidence has been presented that demonstrates quite convincingly that the growth rate and fate of spontaneous carcinoma of the mammary gland in mice may be significantly influenced by heptyl aldehyde.[1] The effect of liquefaction (both partial and in some cases complete) together with a slower growth rate and complete disappearance of the tumor mass could be brought about by the introduction into the organism of the drug either by the diet or by subcutaneous injection (at areas remote to the spontaneous tumor). One of the limiting factors in the introduction of the material through the diet is the fact that the resulting mixed food becomes so bitter with the larger effective doses that the normal food habits of the mouse may be interfered with. The greatest limiting factor of the injection technic is that local ulceration is sometimes quite pronounced.

---

* This experiment has been made possible by grants from the Anna Fuller Fund and the Jane Coffin Childs Memorial Fund for Medical Research. Acknowledgments are also due to Dr. R. Auchincloss of Columbia University and to Dr. F. R. Greenbaum, Director Pharmaceutical Research, National Drug Company, for their kindness in supplying the material, heptyl aldehyde-sodium bisulphite addition compound, used in this investigation.

[1] Strong, L. C. PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 386.

In a recent paper, it was pointed out that in order to influence malignancy the heptyl aldehyde must be used fresh. It was found that as soon as the heptyl aldehyde-normal diet mix had "aged" even for a few days the efficacy of the drug was greatly diminished. One way of "protecting" the precocious deterioration of the heptyl aldehyde was the addition to it of a small amount of methyl salicylate—that is, to approach more or less the chemical situation in the original true or natural oil of Gaultheria.[2] Since, however, the heptyl aldehyde undergoes oxidation readily, one may prohibit or delay, to a certain extent, this change by either of two methods: (1) the addition of a known anti-oxidant, such as salicylic aldehyde to it, or (2) the combining of it with such a chemical as sodium bisulphite. Both of these suggestions have been tried, but the results obtained on spontaneous cancer with the second method alone will be discussed in the present communication.

Heptyl aldehyde-sodium bisulphite addition product[†] is water soluble. It is fairly stable in the dry state, but when in solution, it comes into contact with the tissues of the body, it slowly gives up its heptyl aldehyde.

Seventy-five female mice of the Strong A strain with spontaneous tumors of the mammary gland have so far been injected with freshly prepared distilled water solutions of heptyl aldehyde-sodium bisulphite addition compound. The mice were divided into 5 groups depending upon the amount of the addition product they received daily. The first group of 5 mice received 2 mg of the material dissolved in 0.20 cc of distilled water; the second group of 5 mice, 3 mg in the same amount of water; the third group of 15 mice, 4 mg; the fourth group of 25 mice, 5 mg; and the fifth group of 25 mice 6 mg. The growth rates of the tumors in the various experimental groups are compared with that for the tumors of 120 control mice of the same strain in Chart 1.

---

[2] Strong, L. C., *Yale J. Biol. and Med.*, 1939, **11**, 207.

[†] The material from National Drug Company was prepared by mixing heptyl aldehyde C.P. and sodium bisulphite C.P. together in equimolecular proportions. Upon stirring, the mixture became solid in from 5 to 10 minutes. It was then filtered and dried. The compound, thus formed, is of a brilliant mother-of-pearl lustre and appears to be crystalline. It contains 52.3% of heptylaldehyde. The sample from R. Auchincloss was prepared according to Cumming, Hopper and Wheeler (Systematic Organic Chemistry. Modern Methods of Preparation and Estimation, p. 518) from a reactive solution of $NaHSO_3$. Fifteen grams of Na_2CO_3 were put into 100 cc H_2O. SO_2 was bubbled through. This gave a white precipitate. The SO_2 was passed 50 minutes when solution was cleared of white precipitate. At this time the solution did not yet smell of SO_2. After another 25 minutes the evolution of CO_2 stopped and the solution smelled of SO_2. 18.8 cc heptaldehyde (Eastman) was then added and shortly later another 18.8 cc (100% excess). White precipitate was formed quickly. It was left in the ice box overnight. The material was then filtered and the crystals were washed with a small amount of 95% alcohol. A micro sulfur determination was 13.9%; theoretical 14.6%. The resulting compound is water soluble and is stable in solution at neutral pH.

All injections were done at sites remote to the spontaneous tumors. The variable factors involved in the tolerance to, and the physiological effect of the drug are (1) age of the mouse, (2) body weight, (3) physical condition of the mouse, (4) size of the tumor, and (5) individual peculiarities of both the mouse and certain tumors. The dosage administered to each mouse was carefully determined each day and was altered according to the above variable criteria. The idea used throughout was to inject the maximum effective dose

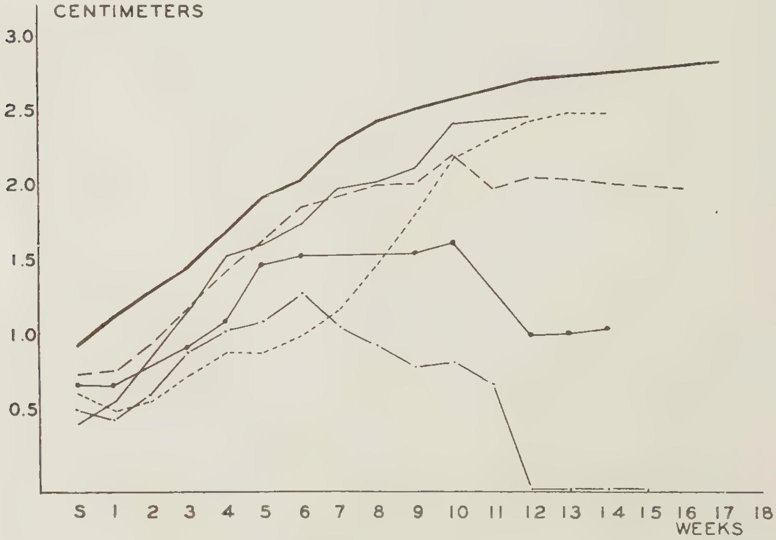


CHART 1.

Chart 1 presents data obtained on the growth rate of spontaneous tumors of the mammary gland in mice on: (a) 120 control mice (heavy solid line); (b) 5 mice receiving 2 mg per day heptyl aldehyde-sodium bisulphite addition product (light solid line); (c) 5 mice receiving 3 mg daily of the same drug (short dash line); (d) 15 mice receiving 4 mg per day of the same drug (long dash line); (e) 25 mice getting 5 mg per day (solid ball and solid line); and (f) 25 mice getting 6 mg per day (dot and broken line).

without interfering with the body weight or physical condition of the mouse.

By an inspection of the chart it may be seen that there is an indirect correlation between the growth rate of spontaneous tumors and the amount of heptyl aldehyde-sodium bisulphite addition compound injected. The same large areas of liquefaction were found here as were obtained by the use of heptyl aldehyde alone. Complete disappearance of some of the spontaneous tumors (6 out of 25 in the fifth series) did occur when the level of 6 mg per day was reached. There have been no recurrences in these cases and the mice are living in normal health at the present time.

A mouse, in which a large tumor is breaking down and being eliminated, is in a critical stage. Some of them die during this process primarily by hemorrhage from the surface of the tumor. This untoward result may be prevented by (1) clipping the hair over the tumor, (2) a periodic bathing of the tumor in 70% alcohol, (3) a drainage by sterile puncture and (4) packing a delicate or thin layer of sterile cotton over a potential "break" in the surface of the tumor. So far the largest tumor that has completely regressed in this series on the chemotherapy of cancer was one that measured 1.5 cm in longest linear diameter.

Heptyl aldehyde-sodium bisulphite addition compound has the further advantage over heptyl aldehyde alone by the fact that local ulceration is considerably reduced.

Experiments with other series of mice receiving larger daily doses of heptyl aldehyde-sodium bisulphite addition compound are being done.

11287 P

Production of Cirrhosis of the Liver of the Normal Dog by High Fat Diets.*

I. L. CHAIKOFF AND C. L. CONNOR.

From the Divisions of Physiology and Pathology, University of California Medical School, Berkeley and San Francisco

During the course of a study on the maintenance of the completely depancreatized dog, it was observed that cirrhosis of the liver developed in 8 of the 16 animals that had been kept alive with insulin for periods between 2.6 and 5.5 years.[1] Infection and obstruction of the extrahepatic bile passages were ruled out as factors that might have induced the scarring of the liver. The animals received a diet containing lean meat, sucrose, bone ash and vitamin supplements and despite its completeness with respect to calories, proteins, vitamins and salts it has been demonstrated repeatedly that this diet induces fatty livers in depancreatized dogs. Although it was recognized that the absence *per se* of the pancreas might be involved, it was nevertheless suggested that an important causative factor in the production of the hepatic fibrosis was the excessive deposition of fat which appeared in the liver during the first few months after pancreatectomy and remained there for long periods.

In view of the importance of the above findings, it seemed highly desirable to determine whether cirrhosis could be induced in the fatty liver of the normal dog. The observations recorded below establish for the first time that the continued presence of excessively high amounts of fat in the liver for several months stimulates hepatic fibrosis even in normal dogs to the extent that it may be called a true cirrhosis, and cause the death of the animals.

Experimental. Well nourished dogs were selected for use in this experiment. Throughout the periods of observation they were maintained on a high fat diet that contained 10 g of lard and 7 g of lean meat per kilo per day. They were fed twice daily by stomach tube when they would not eat, at 7:00 a.m. and at 2.30 p.m. Each animal also received once daily 1 g of Cowgill's salt mixture,[2] 1-5 g of bone ash and vitamin supplements in the form of cod liver oil and a rice bran concentrate.

Four dogs died after being maintained on this diet for 138, 246,

---

* Aided by a grant from the Christine Breon Fund for Medical Research.

[1] Chaikoff, I. L., Connor, C. L., and Biskind, G. R., *Am. J. Path.*, 1938, **14**, 101.

[2] Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 725.

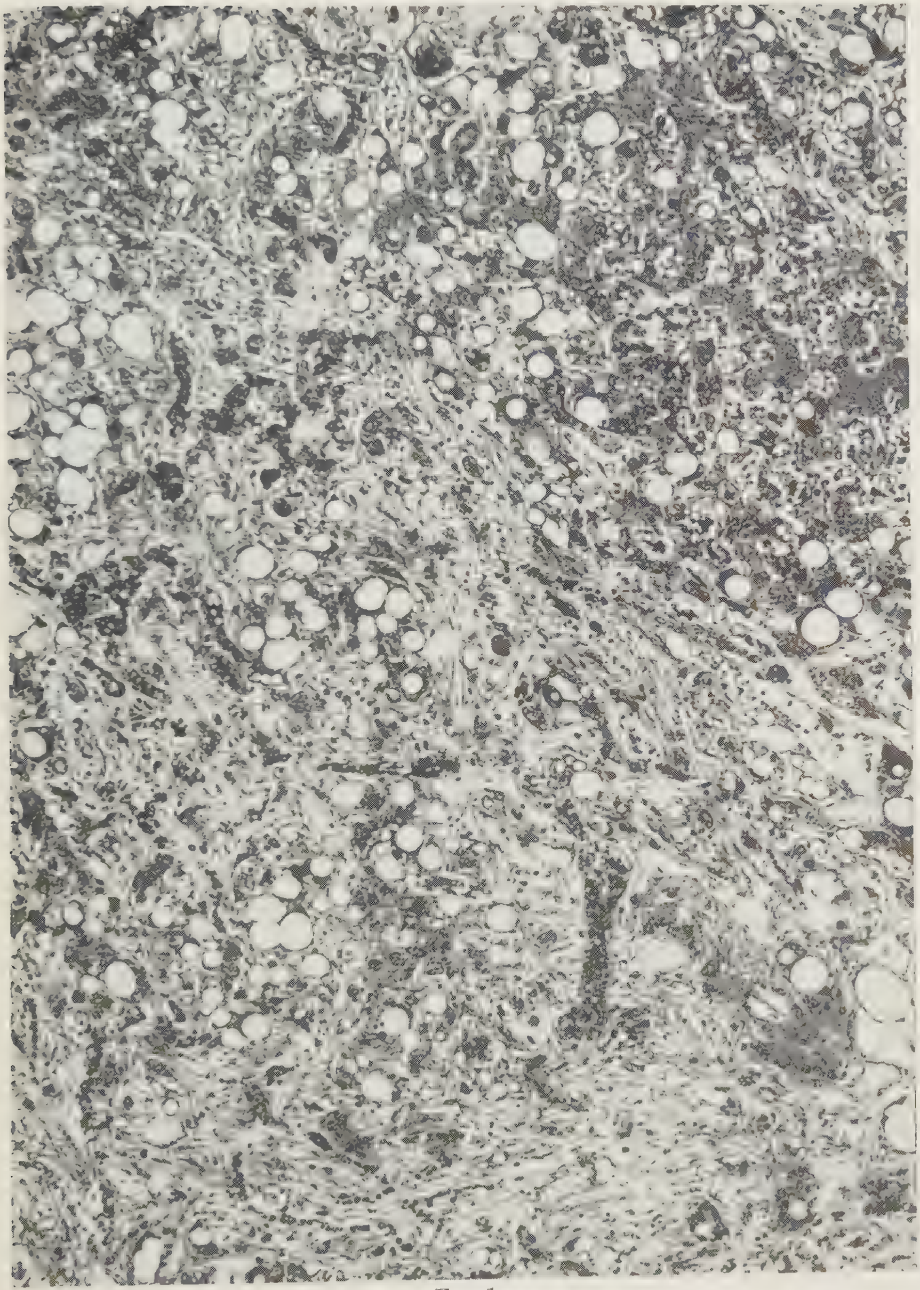


FIG. 1.

Microphotograph of sections stained with Mallory's aniline blue collagen stain. At a magnification at about 170X the uniform distribution of fibrous tissue, fat, and degenerating liver cells can be seen. Many large sinuses are engorged with blood.

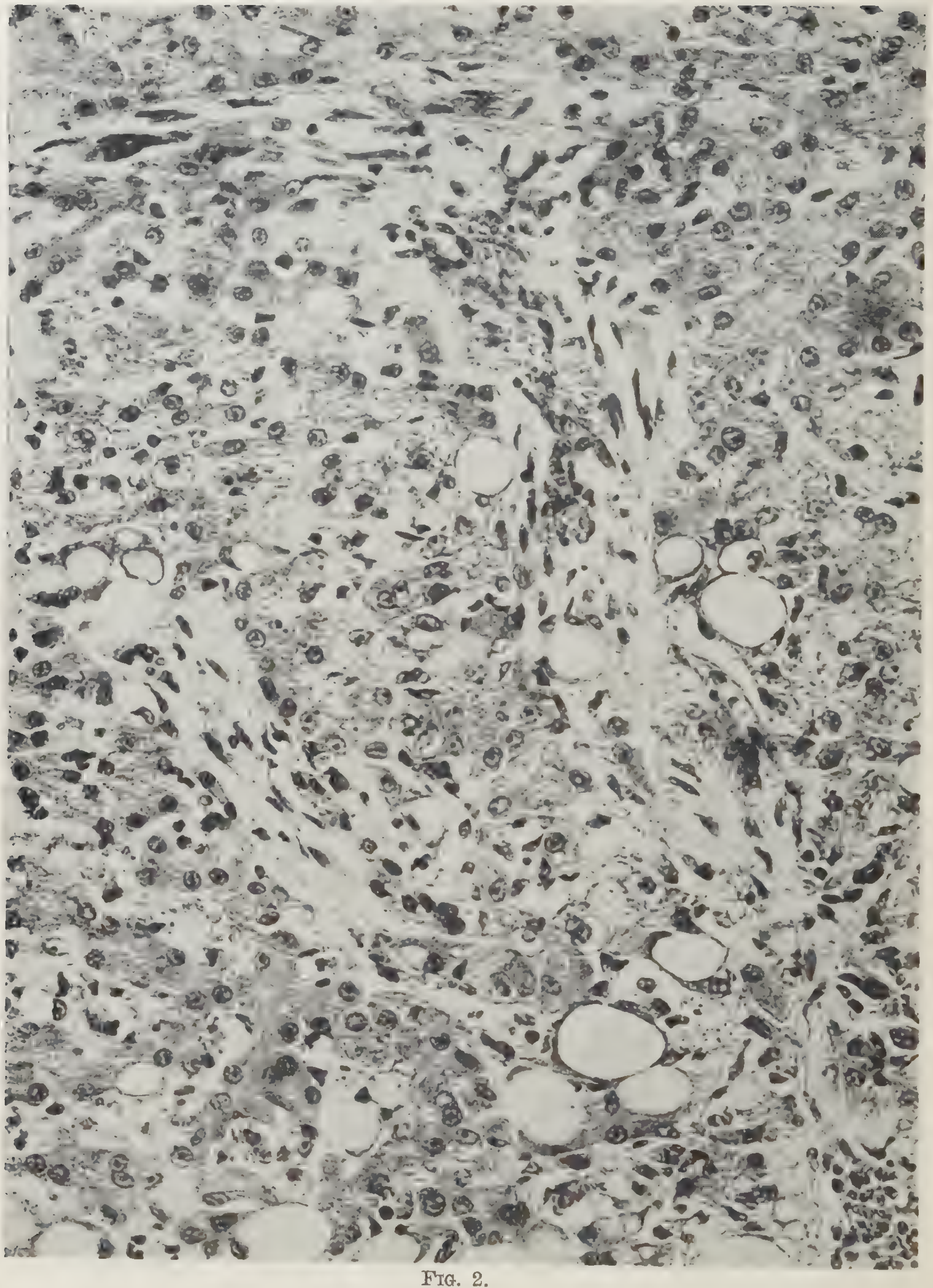


FIG. 2.

Microphotograph of sections stained with Mallory's aniline blue collagen stain. At about 350X, more details of the proliferating fibrous tissue can be seen. In Figs. 1 and 2 the lack of portal or perilobular distribution of the process is evident.

298, and 386 days. The livers of 3 of these animals showed a severe diffuse fibrosis and in all of them a severe fatty infiltration was observed. A typical protocol follows:

Dog F41 was placed on the high fat diet on November 29, 1938, and died December 20, 1939. Its weight at the start was 11.2 kg and at death 12.8 kg. It attained a maximum weight of 16.5 kg during the course of the experiment. The liver weighed 695 g. It was uniformly green in color, firm and rubbery in consistency. It cut with resistance and was lobulated on the surface.

Microscopically (Figs. 1 and 2) the liver showed marked fibrosis and a fatty infiltration. The liver cords were broken up into fragments and in no place had the original pattern of the liver been retained. Fragments of cells were found between proliferating strands of connective tissue that were growing in all directions but had no particular distribution with respect to portal areas or central veins. Blood sinuses were engorged and appeared like fibrous walled blood vessels rather than sinusoids. Some bile duct proliferation had taken place but their duct cells were difficult to recognize because they, too, were enmeshed in connective tissue. Many plugs of inspissated bile were present in large and small masses.

It is of interest to note that the diffuse character of the fibrosis reported here distinguishes this type of cirrhosis from that previously described for dogs receiving alcohol,[3] although the outstanding anatomical change preceding the fibrosis in both instances, as well as in the depancreatized dogs, was severe fatty infiltration of the liver.

11288 P

Immediate Generalized Skin Reactions in Hypersensitive Guinea Pigs.*

J. L. JACOBS. (Introduced by V. Menkin.)

From the Department of Pathology and Bacteriology, Tufts College Medical School, Boston, Mass.

Immediate generalized skin-reactions following contact with or ingestion of allergens have been observed in humans but not hitherto in animals, although Dienes and Simon[1] reported a generalized

---

[3] Connor, C. L., and Chaikoff, I. L., PROC. SOC. EXP. BIOL AND MED., 1938, **39**, 356.

* This investigation was aided by a grant from the Charlton Research Fund of the Tufts College Medical School.

[1] Dienes, L. and Simon, F. A., *J. Immunol.*, 1935, **28**, 321.

delayed reaction in guinea pigs treated with human serum. No observations of generalized skin-reactions in animals, due to simple chemical substances, have been noted in the literature. It seemed of interest, therefore, to report recent observations of such effects in a number of guinea pigs sensitized to citraconic anhydride, together with the sensitization of guinea pigs to new anhydrides having similar properties.

The animals were rendered hypersensitive to citraconic anhydride by a method previously described[2] and were tested by scratching the skin through a 25% dioxane solution of the same substance, usually at intervals of 2 weeks.

It was regularly observed that the more hypersensitive the guinea pig the quicker the onset of the reaction. Animals developing generalized reactions showed within a very few minutes a large local wheal and erythema surrounding the scratch, often several centimeters in diameter. At the same time in both contiguous and remote parts of the skin some pigs showed a diffuse pinkishness apparently accompanied by slight thickening, while others exhibited discrete, urticaria-like wheals with distinct elevation and pinkness. Often some of the old injection-sites on the back flared up at the same time, with marked wheal-formation. The erythema reached a maximum 20 to 30 minutes after the test, following which it gradually faded. Edema associated with reactivated sites and other lesions distant from the original scratch increased for about 2 hours and then also diminished. By the next day all such subsidiary reactions had completely disappeared. In striking, possibly significant contrast to this, the reaction in the vicinity of the scratch after receding to a minimal color and edema in 4 to 5 hours, gradually increased in pinkishness and induration to a second maximum reached about 24 hours after the test. This secondary or delayed reaction at the original site slowly disappeared in the course of several more days. At no time were there any definite symptoms of anaphylaxis although coughs and shivering were occasionally observed. The mechanism of these effects requires further study.

These phenomena invite comparison with the occasional severe generalized skin-reactions from drugs observed in humans. Practically all guinea pigs treated with citraconic anhydride become hypersensitive but in our experiments only 6 out of 61, about 10%, gave generalized reactions. Other pigs less strongly sensitized, however, produced similar but less intense reactions, the subsidiary lesions being fewer in number and not present on areas far from the scratch. Unsensitized control animals were quite negative.

---

[2] Jacobs, J. L., Golden, T. S. and Kelley, J. J., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 74.

In small-scale experiments with 2 new substances, maleic and acetic anhydrides, using the same technic as with citraconic anhydride,[2] extensive immediate wheal-and-erythema reactions were produced, with subsidiary reactions in some cases. With maleic anhydride such isolated reactions appeared many centimeters from the site of scratch. It would seem profitable to examine, on a larger scale, the capacity of these compounds to produce generalized reactions.

Excellent sensitization-effects have been produced by the same method with a third substance, propionic anhydride, using patch-tests with a 25% dioxane solution. Scratch-tests, however, did not yield definite wheal-and-erythema reactions, which is interesting in view of the close chemical relationship between this compound and acetic anhydride.

11289 P

Solubility of Fluorosed Enamel and Dentine.

J. F. Volker.* (Introduced by H. C. Hodge.)

From the Department of Biochemistry and Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The reduction of caries incidence in humans and rats ingesting fluorine may be the result of a decreased acid solubility due to the incorporation of fluorine in the dental tissues. Support for this belief is apparent in the finding of more fluorine in caries-resistant than carious enamel and dentine.[1] If this assumption is correct, we might expect the greatest reduction in acid solubility in the enamel and dentin from mottled teeth since it has been shown that these teeth contain the greatest amounts of fluorine.[2]

The acid solubility of normal and fluorosed rat teeth was studied. The upper incisor teeth were used in all cases. The fluorosed human teeth were obtained from areas where dental fluorosis is endemic and showed a mild degree of "mottling". The fluorosed rat incisors represented were of 2 grades, (1) *mild,* produced by a diet containing 20 p.p.m. of dietary fluorine and (2) *severe,* produced by a diet containing 300 p.p.m. of fluorine.

---

* Carnegie Dental Fellow.

[1] Armstrong, W. D., and Brekhus, P. J., *J. Dent. Res.*, 1938, **17**, 339.

[2] Armstrong, W. D., and Brekhus, P. J., *J. Dent. Res.*, 1938, **17**, 27.

The teeth to be tested were powdered to pass a 100 mesh screen. Separation and purification of the enamel and dentin was accomplished by the centrifugation-flotation method.[3] Solubilities of the fluorosed and normal samples were compared by measuring the weight losses of duplicate 50 mg samples after definite time intervals in 20 cc amounts of .2 M acetic acid/sodium acetate buffer at pH 4.0.[4] Whenever sufficient amounts of the experimental materials were available, measurements were recorded at varying intervals up to and including one hour. Where the supply of experimental material was limited, quadruplicate solubility determinations were made at the end of one hour.

Results. The experimental curves for normal and fluorosed rat (300 p.p.m.) and normal human enamels and dentins may be seen in Fig. 1. Each point represents an average of 3 determinations. The solubilities of normal human and rat enamel are similar, as are those

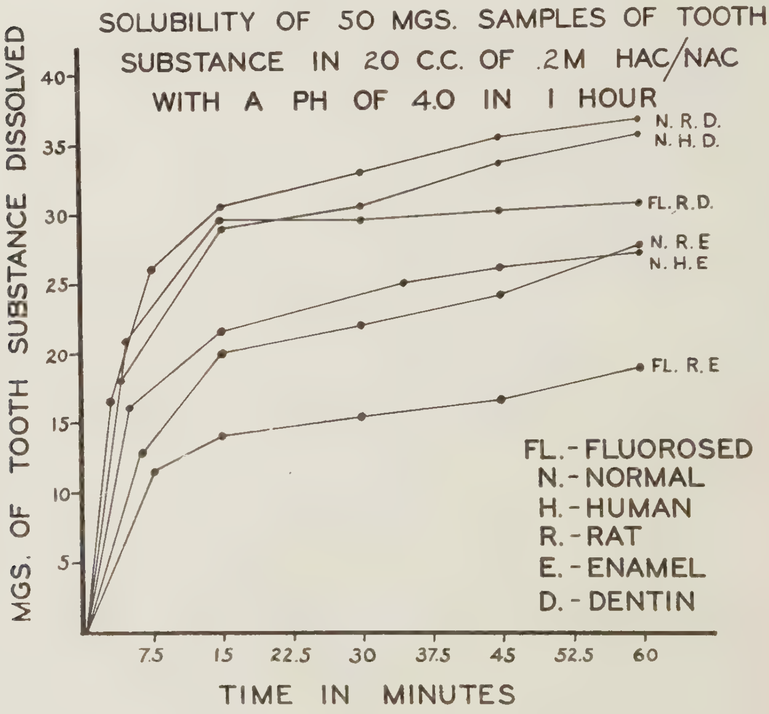


FIG. 1.

---

[3] Manly, R. S., and Hodge, H. C., *J. Dent. Res.*, 1939, **18**, 133.

[4] Volker, J., Thesis, University of Rochester, Rochester, N. Y., 1939.

of normal human and rat dentin. In each case the dentin is considerably more soluble than the enamel. Severely fluorosed rat (300 p.p.m) enamel and dentin have significantly reduced acid solubilities. Both the enamel and dentin solubility values of the mildly fluorosed rat (20 p.p.m.) tissues are comparable to those found in the control tissues (27.1 mg of enamel and 37.8 mg of dentin being lost in one hour). The weight loss of the mildly fluorosed human enamel samples for a one-hour period (26.9 mg) closely approximates that of the normal human enamel (26.6 mg), but the fluorosed human dentin shows a decreased solubility (32.1 mg) when compared with normal human dentin (37.8).

Comment. The findings indicate that the fluorine content of the tooth modifies its solubility. This is not surprising since the presence of fluoride decreases the solubility of related calcium phosphate.[5] Fluorosed rat enamel (300 p.p.m.) containing .125% fluorine was less soluble than normal rat enamel containing .008% fluorine.[6] Parallels are also noted with fluorosed rat dentin (300 p.p.m.) having .25% fluorine and normal rat dentin having only .01% fluorine and with fluorosed human dentin having .07%[7] fluorine and normal human dentin having only .017% fluorine.[2] The inability to demonstrate any reduction in solubility of mildly fluorosed human enamel and rat enamel and dentin (20 p.p.m.) may be explained by the low fluorine content of these tissues.

Conclusion. The presence of fluorine in large amounts may decrease the solubility of the dental hard tissues. Small amounts of fluorine show no demonstrable reduction in enamel solubility. It seems doubtful that the amounts of fluorine present in slightly fluorosed teeth is sufficient to alter their acid solubility.

The kindness of Drs. G. J. Cox, H. C. Hodge and S. B. Finn in supplying the rat teeth and Dr. W. Armstrong in supplying the human teeth used in this study is acknowledged.

---

[5] MacIntire, W. H., anad Hammond, J. W., *Ind. and Eng. Chem.*, 1930, **30**, 160.

[6] Hodge, H. C., Luce-Clausen, E. M., and Brown, E. F., *J. Nutrition*, 1939, **17**, 333.

[7] Bowes, J. H., and Murry, M. M., *Brit. Dent. J.*, 1936, **60**, 556.

11290 P

Preparation and Immunological Properties of Acid-Denatured Egg Albumin.*

CATHERINE F. C. MACPHERSON AND MICHAEL HEIDELBERGER.

From the Departments of Biochemistry and Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

Aqueous solutions of 3 times recrystallized Ea were allowed to stand at room temperature for 1 to 2 days with HCl between pH 1.5 and 2.3. Depending upon the acidity, from 25 to 75% of the Ea was denatured. The DnEa was precipitated by adding NaOH until the isoelectric range, pH 5.2 to 5.4, was reached. Three additional reprecipitations usually sufficed to remove unchanged Ea. The pre-

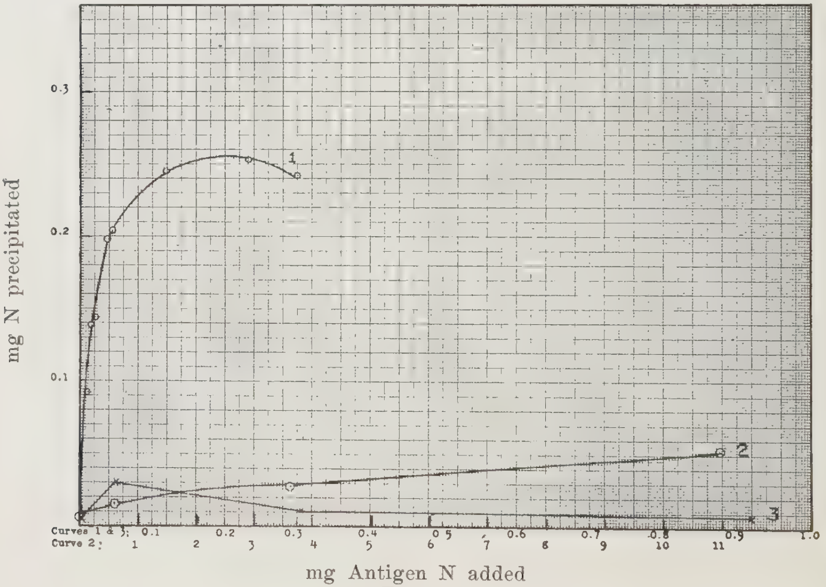


FIG. 1.

Precipitation of Anti-DnEa and Anti-Ea Sera by DnEa and Ea.

Curve 1—N precipitated from anti-DnEa by DnEa. The Ea-anti-Ea curve is similar.[1]

Curve 2—N precipitated from anti-DnEa by Ea.

Curve 3—N precipitated from anti-Ea by DnEa. Supernatants in this series remained opalescent.

Sera compared at approximately equal antibody-content.

---

* Egg albumin and acid-denatured egg albumin are subsequently referred to as Ea and DnEa respectively. This note is from a dissertation to be submitted by Catherine MacPherson in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

cipitate was dispersed in water, dissolved at pH 7, and allowed to stand in the cold for 4 to 5 days after which it was more stable to physiological concentrations of NaCl. Reversion to Ea did not take place.

Three preparations which differed in appearance and solubility in water at pH 7 were found to remove, per mg of DnEa N, the same amount of N from a calibrated anti-DnEa serum.

The reaction between DnEa and its homologous antibody is similar to that between Ea and anti-Ea.[1] The cross-reactions between anti-Ea and DnEa and between anti-DnEa and Ea differ greatly from both homologous reactions and from each other, as shown in Fig. 1.

A study of the chemical and physical properties of Ea denatured in various ways and a correlation of these properties with the immunological findings is now in progress. Previous work in this field appears to have been confined to a preliminary note by Wu, TenBroeck, and Li,[2] the studies of Flosdorff and Chambers[3] and Blumenthal,[4] and the work of Porter and Pappenheimer,[5] and Landsteiner and Rothen[6] on surface-films.

---

[1] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, **62**, 697.

[2] Wu, H., Ten Broeck, C., and Li, C. P., PROC. SOC. EXP. BIOL. AND MED., 1926-7, **24**, 472.

[3] Flosdorff, E. W., and Chambers, L. H., *J. Immunol.*, 1935, **28**, 297.

[4] Blumenthal, D., *J. Biol. Chem.*, 1936, **113**, 433.

[5] Porter, E. F., and Pappenheimer, A. M., Jr., *J. Exp. Med.*, 1939, **69**, 755.

[6] Landsteiner, K., and Rothen, A., *Science*, 1939, **90**, 65.

11291 P

Improved Sequence for Rapid Consistent Purification of Equine Encephalomyelitis Virus Protein.*

A. R. TAYLOR, D. G. SHARP, HAROLD FINKELSTEIN, DOROTHY BEARD AND J. W. BEARD.

From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

The procedure described here for purification of equine encephalomyelitis virus protein[1] yields consistently a product of high molecular homogeneity. Advantage is taken chiefly of the following principles: (1) prolonged extraction to aid in eliminating the normal chick tissue component;[2] (2) extraction, fractionation and solution of the protein in a balanced salt solution, mammalian Ringer,[3] instead of 0.9% NaCl or buffer salt solutions; (3) filtration of crude extracts with celite to remove mucoid and colloid materials; and (4) aggregation or partial precipitation of the protein in slightly acid medium prior to the first ultracentrifugal cycle.

Diseased embryo tissue ground in the cold in the usual way,[2] is suspended in 4 times its volume of normal Ringer's solution made to pH 9.0 with NH_4OH without buffer salts. After extraction at 5°C for 72 to 96 hours, gross material is eliminated in the angle centrifuge. To each 100 cc of the turbid supernatant fluid, 5 g of No. 512 Celite Filter Aid (Johns-Manville Co., N. Y.) are added and the suspension is filtered with suction through a 1 to 2 mm mat of No. 503 Celite. Standard celite then added to the filtrate, 2 g per 100 cc, is filtered off through a mat also of standard celite. Sometimes the latter step is repeated to obtain an entirely clear filtrate.

The filtrate is acidified to pH 6.5 with 0.2 N HCl, and 120 cc of it distributed immediately in 8 collodion tubes is spun at 17,000 g for 45 minutes. The pellets are taken up in 30 cc of Ringer's solution. In 2 tubes, the solution is spun at 17,000 g for 5 minutes. The supernatant fluid diluted to 60 cc is ultracentrifuged 30 minutes at 67,000 g and the resulting pellets are taken up in 15.0 cc of Ringer fluid.

---

* This work was aided by the Dorothy Beard Research Fund and by a grant from Lederle Laboratories, Pearl River, New York.

[1] Taylor, A. R., Sharp, D. G., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 462.

[2] Sharp, D. G., Taylor, A. R., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 459.

[3] Bayliss, W. M., *Principles of Physiology*, Longmans, Green and Co., Ltd., London, 4th ed., page 211.

This is spun 15 minutes at 15,000 g and the supernatant fluid diluted to 30 cc. From this volume, the protein is sedimented at 67,000 g for 30 minutes. The 2 pellets are dissolved in 1.5 to 3 cc of Ringer's solution, pH 9.0 depending on yield.

The character of the product with respect to homogeneity can be judged by the photometric tracing in Fig. 1. Practically all of the normal chick component[2] is eliminated in the first ultracentrifugal cycle and no evidence of it has been observed after the second cycle. Similar loss of colloid occurs in the process of filtration and in the first and second ultracentrifugal cycles.

Only slight loss of infectivity occurs in filtration, and no evidence is seen of damage to the protein by short exposure to pH 6.5. Precipitated at pH 6.5 the purified protein redissolves to its initial homogeneity and infectivity. Acidification facilitates sedimentation and separation of the protein from the normal chick component in the first relatively low speed cycle and greatly increases the final yield of the homogeneous product, ca 0.4 mg per gram of diseased embryo

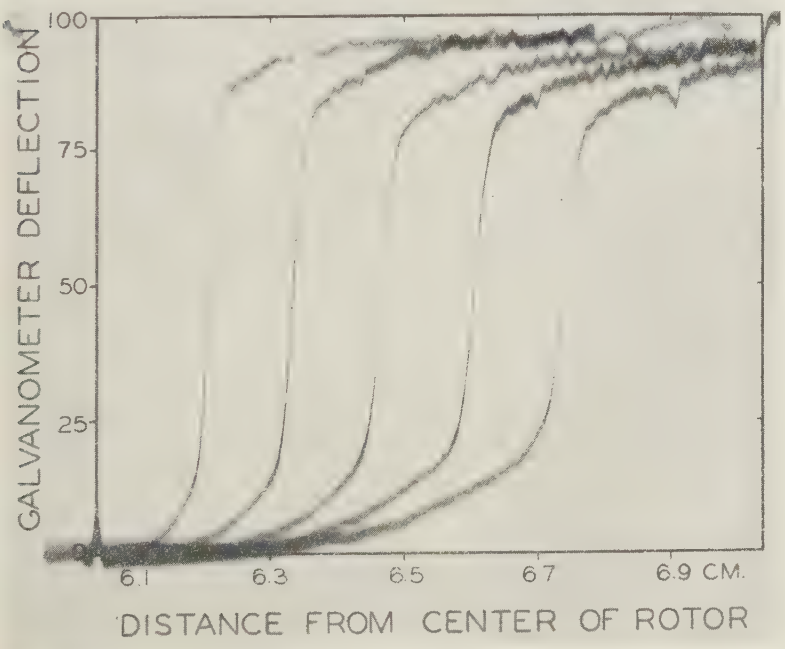


FIG. 1.

Photometer curves from sedimentation diagrams of a typical preparation of Eastern strain equine encephalomyelitis virus protein purified by the method described. The protein concentration was 1.8 mg per cc, the interval between curves 5 minutes and the ultracentrifugal field 17,000 g.

tissue. In Ringer's solution, at a protein concentration of 1 to 1.5 mg per cc, infectivity does not diminish within 7 days at 5°C and the sedimentation pattern is unchanged for periods of several weeks. In 0.9% NaCl or Ringer-buffer salt solution (NH_4OH-NH_4Cl, 0.05 M), however the protein loses much infectivity in 96 hours. In the latter solution the boundary may remain intact for 3 weeks when infectivity has diminished 6 decimal dilutions or more.

11292 P

Immunization with Non-Infectious Formalin Derivative of Purified Equine Encephalomyelitis Virus Protein.*

D. G. SHARP, A. R. TAYLOR. HAROLD FINKELSTEIN, DOROTHY BEARD AND J. W. BEARD.

From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

Previous ultracentrifugal studies[1] of crude equine encephalomyelitis chick vaccines[2] have failed to yield definite evidence of the character of the immunizing principle. Information concerning its probable nature has been sought in the present work by study of the purified virus protein treated with formaldehyde.

Purified protein (Eastern strain),[3] dissolved in Ringer's solution, pH 8-9, 2.0 mg per cc, was treated with various concentrations of CH_2O. Before exposure to CH_2O, one m.i.u.[4] of protein was $10^{-13.5}$ g. When the concentration of CH_2O was less than 0.01 M, inactivation was not always complete in 2 weeks, and tests for immunizing capacity were not made. With other concentrations of CH_2O used, inactivation was complete in the time shown in Table I, as judged by the failure of $10^{-5.5}$ g of protein to infect mice.

The effect of CH_2O on the protein molecules was somewhat similar

---

* This work was supported by a grant from Lederle Laboratories, Pearl River, N. Y., and by aid from The Dorothy Beard Research Fund.

[1] Taylor, A. R., Sharp, D. G., Finkelstein, H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 462.

[2] Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 490.

[3] Taylor, A. R., Sharp, D. G., Finkelstein, H., Beard, D., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 648.

[4] Cox, H. R., and Olitsky, P. K., *J. Exp. Med.*, 1936, **63**, 745.

TABLE I.
Immunization of Guinea Pigs with the Non-Infectious Formaldehyde-treated Equine Encephalomyelitis Virus Protein.

CH_2O molar	Inactivation time, days	pH	Total protein* per guinea pig, mg	Test for immunity†	
				Vaccinated	Control
.01	7	7.0	.125	3/3‡	0/5
			.250	6/6	”
.02	4	6.5	.125	4/4	”
			.250	5/6	”
.04	3	6.3	.125	3/4	”
			.250	4/6	”

*Given subcutaneously in 2 equal doses in 0.1 cc at interval of 1 week.
†500 to 1,000 mouse infectious units of virus given intracerebrally.
‡Numerator = survivals; denominator = No. of test guinea pigs.

to its action on tobacco mosaic virus protein.[5] With CH_2O 0.01 M or less, the protein remained in solution but lost slightly in homogeneity, showing a slightly diffuse boundary with a sedimentation pattern similar to that of the untreated protein[3] persisting with little change for more than 3 weeks. In 0.02 M and higher concentrations of CH_2O, the protein rapidly became quite inhomogeneous and increasingly insoluble, losing the definite boundary in 2-4 days. In no instance was there evidence of molecular disruption to small soluble protein fragments of uniform size, nor was there evidence of the component $S_{20°} =$ ca 60×10^{-13} cm sec^{-1} $dynes^{-1}$ previously described.[6]

The non-infectious, soluble though slightly inhomogeneous protein derivative with 0.01 M CH_2O has immunized all guinea pigs receiving it, as shown in the typical experiment in Table I. The more inhomogeneous fragments resulting from 0.02 M and higher concentrations of CH_2O definitely immunize less well.

The results show that the immunizing material derived with CH_2O from the pure protein consists of an inhomogeneous group of degradation products nearly the size of the intact protein or somewhat smaller. With time, as the CH_2O reacts with the protein, there is a progressive increase in inhomogeneity and insolubility of the products when the pH is allowed to drop from the initial value of 8-9 to pH 7.0 to 6.3, as is shown in Table I. It is probable that the immunizing material derived with CH_2O from the virus in crude vaccines is of the same nature, and this probability has been emphasized by the isolation of such a material with a definite, though slightly diffuse boundary from crude vaccines less than 48 hours old (infectivity diminished at least 5 decimal dilutions). Older vaccines fractionated at pH below 7.0, however, yield materials that show only suggestions of a boundary or completely diffuse shadows in the sedimentation pattern where boundaries corresponding to this approximate particle size should appear.

---

[5] Wyckoff, R. W. G., Biscoe, J., and Stanley, W. M., *J. Biol. Chem.*, 1937, **117**, 57.

[6] Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G., *Science*, 1938, **87**, 89.

11293

Water-Soluble Antihemorrhagic Compounds.

S. ANSBACHER, ERHARD FERNHOLZ AND M. A. DOLLIVER.*

From The Squibb Institute for Medical Research, New Brunswick, N. J.

The interest in vitamin K analogues suitable for parenteral medication is evident from the current literature. Usually patients with hypoprothrombinemia due to obstructive jaundice receive orally vitamin K and bile or bile salts. Frequently these latter substances are badly tolerated and it seems that they are not needed, if the antihemorrhagic material is administered parenterally. In cases of severe hepatic insufficiency vitamin K orally is apparently not beneficial, but intravenous injections of a vitamin K analogue, such as e.g. 2-methyl-1,4-naphthoquinone, may be effective in raising the prothrombin content of the blood, as reported by Rhoads and Fliegelman.[1]

The first synthetic antihemorrhagic substance to be employed parenterally in the clinic was phthiocol.[2, 3] Relatively large doses (50-100 mg) are required, because the potency of this compound is small.[4] Flynn and Warner[5] determined that phthiocol had but 1/500 the activity of methylnaphthoquinone, and we had reported previously[6] an activity ratio of 1 :1000 and even of 1 :4000, when an especially purified sample of synthetic phthiocol was used.

---

* Chemist at the Development Laboratory of E. R. Squibb & Sons, Brooklyn, N. Y.

[1] Rhoads, J. E., and Fliegelman, M. T., *J. Am. Med. Assn.*, 1940, **114**, 400.

[2] Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R., *J. Am. Med. Assn.*, 1939, **113**, 380.

[3] Butt, H. R., Snell, A. M., and Osterberg, A. E., *Proc. Staff Meeting Mayo Clin.*, 1939, **14**, 497.

[4] Ansbacher, S., and Fernholz, E., *J. Am. Chem. Soc.*, 1939, **61**, 1924.

[5] Flynn, J. E., and Warner, E. D., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 190.

As already mentioned, methylnaphthoquinone in an aqueous medium is being employed clinically for intravenous medication. Aqueous or physiologic salt solutions containing 1 mg in 10 ml appear to be stable when kept in a refrigerator, and methylnaphthohydroquinone is even more soluble (3 to 4 times) in water. Recently Dann[7] found that oil solutions are roughly 3 times as active as aqueous ones. Our assays (Table I) show that methylnaphthoquinone, when administered orally, is about twice as potent in water as in oil, and confirm our previous observation[8] that the oil, used as a medium for administering vitamin K, interferes to a noticeable degree with the potency. Intravenously the aqueous solution is about as active as the oil solution orally.

The sodium salt of 2-methyl-1,4-naphthohydroquinone disulfuric acid was synthesized by Fieser and Fry[9] who stated that it showed antihemorrhagic activity at a level of 2γ. We found (Table I) that this compound has a potency of 1 unit in about 25γ, and 5γ is fully effective in an 18-hour test (Table II).

Sodium 2-methyl-1,4-naphthohydroquinone diphosphate was prepared by Fieser and Fry[9] and by Foster, *et al.*[10] The latter authors stated that it had an oral effectiveness lying below 2γ. Our data (Table I) show that this substance has a potency of 1 unit in about 10γ, and 5γ is the minimum fully effective dose when the test period is prolonged to 18 hours (Table II). Compared with methylnaphthoquinone, this compound is but 1/20 as active. The results presented in Tables I and II do not agree with those of Foster, *et al.*,[10] who expressed the opinion that this sodium salt represents probably the most active antihemorrhagic substance known when compared on a molecular basis with methylnaphthoquinone. Furthermore, our observations do not support the speculation of these investigators as to whether the antihemorrhagic effects of 2-methyl-1,4-naphthoquinone, of its reduced form, and of like substances, are not mediated through a phosphoric ester.

We wish to report on still another water-soluble antihemorrhagic compound, 2-methyl-1,4-naphthylene-dioxy diacetic acid. It has a potency of 1 unit in about 2 mg (Table I), and 100γ is effective in

---

[6] Fernholz, E., and Ansbacher, S., *Science*, 1939, **90**, 315.

[7] Dann, F. P., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 663.

[8] Ansbacher, S., *J. Nutrition*, 1939, **17**, 303.

[9] Fieser, L. F., and Fry, E. M., *J. Am. Chem. Soc.*, 1940, **62**, 228.

[10] Foster, R. H. K., Lee, J., and Solmssen, U. V., *J. Am. Chem. Soc.*, 1940, **62**, 453.

TABLE I.
Vitamin K Assays.

Substance	Mode of administration	Vehicle	Response* > 1 unit† γ	Response* = 1 unit γ	Response* < 1 unit γ
2-methyl-1,4-naphthoquinone‡	orally	oil	3/4	1/2	1/4
	”	water	1/2	1/4	1/10
	intraven.	” §	3/4	1/2	1/4
Sodium 2-methyl-1,4-naphthohydroquinone diphosphate	orally	”	12.5	10	7.5
Sodium 2-methyl-1,4-naphthohydroquinone disulfate	”	”	30	25	20
Phthiocol	”	capsules	600	500	400
Purified phthiocol	”	”	2250	2000	1750
2-methyl-1,4-naphthylene-dioxy diacetic acid	”	water	2250	2000	1750

*A minimum of twenty severely vitamin K-deficient chicks was used for each dose level.
†Ansbacher unit (*J. Nutrition*, 1939, **17**, 303).
‡Methylnaphthoquinone or methylnaphthohydroquinone.
§Water or physiologic salt solution.

TABLE II.
Minimum Effective Doses in 6- and 18-hour Tests.

Substance	6 hr γ	18 hr γ	Ratio
2-methyl-1,4-naphthoquinone*	1/2† 1/4‡	1/4† 1/8‡	2:1
Sodium 2-methyl-1,4-naphthohydroquinone diphosphate	10	5	2:1
Phthiocol (purified) 2-methyl-3-hydroxy-1,4-naphthoquinone	2000	1000	2:1
Sodium 2-methyl-1,4-naphthohydroquinone disulfate	25	5	5:1
Vitamin K_1, 2-methyl-3-phytyl-1,4-naphthoquinone	15	1	15:1
2-methyl-1,4-naphthylene-dioxy diacetic acid	2000	100	20:1

*Methylnaphthoquinone or methylnaphthohydroquinone.
†Orally in oil or intravenously in an aqueous medium.
‡Orally in water.

an 18-hour test (Table II). This substance was prepared in the following manner:

To 15 g of 2-methyl-1,4-naphthohydroquinone and 16.3 g of monochloracetic acid, 13.8 g of sodium hydroxide in 350 ml of water was added, the mixture refluxed for 6 hours under nitrogen and allowed to stand overnight. The product was then diluted with an equal volume of water and 10% hydrochloric acid added until an oil separated which soon solidified. It was removed by filtration, taken up in a 10% sodium carbonate solution, warmed in the presence of a decolorizing charcoal, filtered and reprecipitated with dilute hydrochloric acid. A pinkish powder with a m.p. of 204-10° was thus obtained. It was dissolved in a 10% sodium bicarbonate solution, treated three times with decolorizing charcoal, and reprecipitated from the almost colorless solution with dilute hydrochloric acid. The final product was a white powder, m.p. 217-218°; yield 7.5 g (30%).

Analysis. Calculated for $C_{15}H_{14}O_{16}$: C, 62.04%; H, 4.86%.
Found: C, 61.38%; H, 4.92%.

Other water-soluble vitamin K substitutes have been prepared and studied clinically. According to Butt *et al.*,[11] the 1,4-dihydroxy-2-methyl-3-naphthaldehyde of Binkley and coworkers[12] is effective at a level of 10 mg, and Broun[13] found that the 2-methyl-4-amino-1-naphthol hydrochloride of Doisy and associates[14] has a definite effect at 5 mg. Synthetic vitamin K_1 is practically insoluble in water. It has been used by Frank, *et al.*[15] in form of a colloidal suspension in glucose and was active at 10 mg. These data would seem to confirm the statement by Rhoads and Fliegelman[1] that "2-methyl-1,4-naphthoquinone appears to be the most potent agent for the treatment of prothrombin deficiency so far employed clinically."

Summary. Methylnaphthoquinone or methylnaphthohydroquinone is the most active vitamin K compound known. It is as effective intravenously in an aqueous medium as orally in oil solution. *Per os* its potency is greater in water than in oil. The phosphate derivative is considerably less active. The sulfate is not as potent and not as rapidly absorbed by the organism as the phosphate. A new water-soluble vitamin K-active compound is described.

---

[11] Butt, H. R., Snell, A. M., Osterberg, A. E., and Bollman, J. L., *Proc. Staff Meeting Mayo Clin.*, 1940, **15**, 69.

[12] Binkley, S. B., Cheney, L. C., Holcomb, W. F., MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A., 98th Meeting, Am. Chem. Soc., Boston, Sept. 12, 1939.

[13] Broun, G. O., 12th Ann. Meeting, Central Soc. Clin. Res., Chicago, Nov. 3 and 4, 1939 (*J. Am. Med. Assn.*, 1940, **114**, 440).

[14] Doisy, E. A., MacCorquodale, D. W., Thayer, S. A., Binkley, S. B., and McKee, R. W., Nation. Acad. Sci., Brown U. Meeting, Oct. 23-25, 1939 (*Science*, 1939, **90**, 407).

[15] Frank, H. A., Hurwitz, A., and Seligman, A. M., *New England J. Med.*, 1939, **221**, 975.

11294

Gonadotropic Response to Subcutaneous and Intraperitoneal Injections of Urine Prolan in White Rats.

FELIX SULMAN AND JOACHIM SKLOW. (Introduced by B. Zondek.)
From the Laboratory of the Gynæcological-Obstetrical Department, Rothschild-Hadassah University Hospital, Jerusalem.

Investigations by Collip[1] and Evans[2] showed that hypophyseal gonadotropic hormone is much more effective by subcutaneous than by intraperitoneal administration. With reference to gonadotropic hormone derived from pregnant mare serum (PMS), Evans[2] showed that it was just as effective intraperitoneally as subcutaneously. Later Pencharz[3] demonstrated that intraperitoneally PMS was 2 to 3 times more effective than subcutaneously, the degree of effectiveness, however, depending upon the number of injections.

As to the effectiveness of intraperitoneal and subcutaneous administration of pregnancy urine prolan, Evans[2] reported: "Pregnancy prolan, which contains no antagonist, is distinctly less effective intraperitoneally than subcutaneously. Here the difference seems to be due merely to faster absorption by the intraperitoneal route with consequent increased rate of excretion by the kidney or destruction by the tissues." The following experiments are intended to demonstrate that, provided a certain experimental procedure is used, it is possible to make pregnancy urine prolan develop its full effect also in the intraperitoneal route.

For the experiments, 72 infantile female rats, 3-4 weeks old and weighing 20-30 g were used. They received prolan by various methods, by a single injection, or 3 times at intervals of 24 hours, or 6 times during 40 hours, either intraperitoneally or subcutaneously. Sixty hours after the first injection we began taking vaginal smears every 12 hours, and 120 hours after the first injection the animals were killed and their ovaries inspected.

Two prolan preparations were tested in this way: (1) "Purified prolan" (PP) which contained 333,333 RU per g. (2) Crude prolan (CP) which contained 1000 RU per g. The results of these experiments are shown in Table I.

As may be deduced from the experiments there is no fundamental difference between the effectiveness of purified prolan (PP) and crude prolan (CP). We shall only compare the results read from the estrous reaction (APR I):

---

[1] Collip, *Canad. Med. Assn. J.*, 1936, **34**, 458.

[2] Evans, H. M., Korpi, K., Pencharz, R. I., and Simpson, M. E., *Univ. Calif. Publ. Anat.*, 1936, **1**, 237.

[3] Pencharz, R. I., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 525.

One single injection of prolan was approximately 10 times (in case No. 24 at least 6 times) as effective subcutaneously as intraperitoneally (Nos. 2, 6, 20, 24). When, however, the injections were given in 3 portions the subcutaneous route was only twice as effective as the intraperitoneal one (Nos. 9, 10, 26, 27). When the same amount of prolan was, however, distributed over 6 injections there was no longer any difference between intraperitoneal and subcutaneous administration (Nos. 15 and 32).

Taking the corpus luteum reaction (APR III) as a standard almost the same values were obtained and it even seemed that with 6 intraperitoneal injections (*Cf.* No. 16) the APR III could be produced with smaller doses intraperitoneally than subcutaneously.

Experiments Nos. 15 and 32 represent the routine method of prolan standardization (Zondek[4]) in terms of estrous units using 6 injections. If this experimental technic is adhered to it is therefore irrelevant whether the standardization is performed with subcutaneous or intraperitoneal injections.

Discussion. It was possible to verify the results obtained by Evans[2] that subcutaneous administration of pregnancy urine prolan proved to be more effective than intraperitoneal, provided only 1 to 3 injections were given. When, however, the same amount of prolan was given in 6 portions, both methods were equally effective. We are inclined to attribute these results to the more rapid absorption of the hormone from the intraperitoneal deposits, as explained by Evans.[2] After only one to 3 injections it is very rapidly absorbed and its effectiveness considerably impaired. Only when using 6 injections over 40 hours the absorptional conditions in the peritoneum become equal to those in the subcutis, so that optimal utilization of prolan is possible.

When standardizing small amounts of prolan we had, moreover, the opportunity of studying the following phenomenon, which has been described for PMS by Hamburger:[5] Distribution of 1 RU of prolan over several subcutaneous injections produces a less marked gonadotropic reaction than the administration of one single dose. With larger doses, however, this does not hold true. This phenomenon may be explained by the fact that, by splitting up a threshold dose

---

[4] Zondek, B., *Z. Gebh. and Gyn.*, 1926, **90**, 378; *Med. Klin.*, 1927, No. 13; *Hormone d. Ovariums u. d. Hypophysenvorderlappens*, Springer, Berlin, 1st ed., 1931; *cf.* Zondek, B., and Aschheim, S., *Klin. Wschr.*, 1927, **6**, No. 6.

[5] Hamburger, Ch., *Quart. J. Pharm. and Pharmacol.*, 1938, **11**, 186.

TABLE I.

Exp. No.	Prolan	Injections	Doses Weight	Doses RU*	Gonadotropic reactions (APR) after subcutaneous injection I Estrous	II Folliele hemorrhage	III Corp. lut.	Gonadotropic reactions (APR) after intraperitoneal injection I Estrous	II Folliele hemorrhage	III Corp. lut.
1	PP	1 x	1 gamma	0.3	—	—	—	—	—	—
2			1.5 ’’	0.5	+	—	—	—	—	—
3			3 ’’	1	+	—	—	—	—	—
4			6 ’’	2	+	—	—	—	—	—
5			9 ’’	3	+	—	—	—	—	—
6			15 ’’	5	+	—	—	+	—	—
7	PP	3 x	1 ’’	0.3	—	—	—	—	—	—
8			1.5 ’’	0.5	—	—	—	—	—	—
9			3 ’’	1	+	—	—	—	—	—
10			6 ’’	2	+	—	—	+	—	—
11			9 ’’	3	+	—	—	+	—	—
12			15 ’’	5	+	—	+	+	—	+
13	PP	6 x	1 ’’	0.3	—	—	—	—	—	—
14			1.5 ’’	0.5	—	—	—	—	—	—
15			3 ’’	1	+	—	—	+	—	—
16			6 ’’	2	+	—	—	+	—	+
17			9 ’’	3	+	—	+	+	—	+
18			15 ’’	5	+	—	+	+	—	+
19	CP	1 x	0.5 mg	0.5	—	—	—	—	—	—
20			1 ’’	1	+	—	—	—	—	—
21			1.5 ’’	1.5	+	—	—	—	—	—
22			3 ’’	3	+	—	—	—	—	—
23			4.5 ’’	4.5	+	—	—	—	—	—
24			6 ’’	6	+	—	—	—	—	—

25	CP	3 x	0.5	”	0.5	—	—	—	—	—	—
26			1	”	1	+		—	—	—	—
27			1.5	”	1.5	+	—		+	—	—
28			3	”	3	+			+	—	—
29			4.5	”	4.5	+	—	+	+	—	+
30			6	”	6	+	—	+	+	—	+
31	CP	6 x	0.5	”	0.5	—	—		—	—	—
32			1	”	1	+	—	—	+	—	—
33			1.5	”	1.5	+	—	—	+	—	—
34			3	”	3	+	—	—	+		
35			4.5	”	4.5	+	—	+	+	—	+
36			6	”	6	+	—	+	+	—	+

---

*According to Zondek's definition[4] 1 RU is the smallest amount of prolan which given in 6 injections spread over 36 hours to a 3-4 week-old female, white rat weighing 20-30 g produces the estrous reaction (APR I).

(as 1 RU certainly is) several subthreshold doses are produced which cannot induce the gonadotropic response in the ovary.

Summary. Comparing the gonadotropic response of the organism to subcutaneous or intraperitoneal administration of pregnancy urine prolan the following results were secured: (1) If the minimum dose is split up into 6 portions intraperitoneal and subcutaneous administration are equally effective. (2) If the minimum dose is split up into 3 portions the intraperitoneal route is approximately half as effective as the subcutaneous one. (3) If the minimum dose is given in one injection the intraperitoneal administration is about 1/10 as effective as subcutaneous. (4) Using the subcutaneous standardization method with minute doses as *e.g.*, 1 RU (estrous effect) the gonadotropic effect of 6 subthreshold doses is less marked than that of one single threshold dose.

11295

Cheilosis Successfully Treated with Synthetic Vitamin B_6.

SUSAN GOWER SMITH AND DAVID W. MARTIN. (Introduced by David T. Smith.)

From the Department of Medicine and Department of Pediatrics, Duke University School of Medicine, Durham, N. C.

Aykroyd and Krishnam[1] observed that the incidence of angular stomatitis (sores in the corners of the mouth, perleche, cheilosis) coincided with a deficiency of some factor or factors of the vitamin B_2 complex.

More recently Sebrell and Butler[2] produced these lesions in 10 out of 18 women subsisting on a riboflavin-deficient diet. The lesions developed in a period of 94 to 130 days. They failed to respond to nicotinic acid but responded to riboflavin, the complete healing requiring from 5 to 58 days. Sebrell and Butler[3] added more evidence to their previous findings and these have been confirmed by others.[4, 5, 6]

---

[1] Aykroyd, W. R., and Krishnam, B. G., *Ind. J. Med. Res.*, 1937, **24**, 707.

[2] Sebrell, W. H., and Butler, R. E., *U. S. Pub. Health Rep.*, 1938, **53**, 2882.

[3] Sebrell, W. H., and Butler, R. E., *U. S. Pub. Health Rep.*, 1939, **54**, 2121.

[4] Sydenstricker, V. P., Geeslin, L. E., Templeton, C. M., and Weaver, J. W., *J. A. M. A.*, 1939, **113**, 1697.

[5] Spies, T. D., Vilter, R. W., and Ashe, W. F., *J. A. M. A.*, 1939, **113**, 931.

[6] Jolliffe, N., Fein, H. D., and Rosenblum, L. D., *New Eng. J. Med.*, 1939, **221**, 921.

In view of these facts, it seems important to report our observations on 4 consecutive cases of cheilosis occurring spontaneously in association with other deficiency syndromes in patients admitted to this hospital and treated with synthetic vitamin B_6 [2 methyl, 3 hydroxy, 4.5 di(hydroxymethyl) pyridine].*

The first patient treated was a 7-year-old colored girl admitted to the hospital with a diagnosis of pellagra. Pellagrous lesions were present on the hands and feet. A dietary history revealed a marked deficiency of the vitamin B-complex. There were typical cheilosis lesions of the lips, characterized by maceration and fissuring at the angles. (Fig. 1a.) There was an associated severe anemia. The patient was placed on the basic diet of Smith and Ruffin which is known to be low in the B-complex and especially so in riboflavin.[7] Treatment with synthetic vitamin B_6 hydrochloride† was started with 20 mg intravenously 24 hours after admission. Within 5 hours slight but definite objective improvement could be observed in the mouth lesion. In 24 hours the improvement was demonstrable by

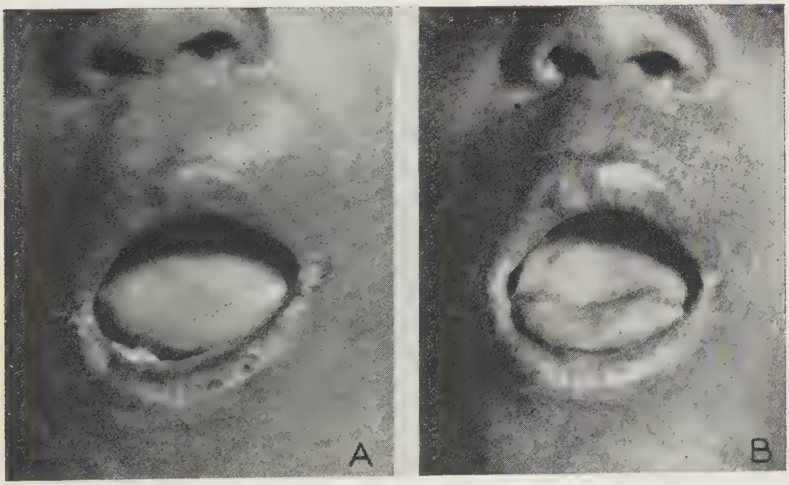


FIG. 1.

A. Before treatment was initiated.
B. Five days after starting treatment with B_6.
(20, 20, and 40 mg given 1st, 2nd, and 4th day respectively).

---

* The synthetic vitamin B_6 used in this work was obtained through the courtesy of Merck and Co., Rahway, N. J.

[7] Smith, D. T., and Ruffin, J. M., *Arch. Int. Med.*, 1937, **59**, 631.

† The vitamin B_6 was prepared for intravenous use by dissolving it in freshly distilled water at a concentration of 50 mg per cc. The vitamin is readily soluble in water and no difficulty is encountered in getting this concentration. The solution was placed in a vaccine bottle and sterilized by autoclaving.

photograph. A 20 mg dose was given intravenously the next day with continued improvement. The next 2 days B_6 therapy was omitted and there was a slight regression. At this time a 40 mg dose was given intravenously and the improvement obtained within 24 hours was marked. (Fig. 1b.) The lesion healed completely from the effects of this dose. This is the only anemia patient studied in which the anemia responded after B_6 treatment, while the patient was subsisting on a diet very low in iron.

The second case of cheilosis occurred in a white woman, aged 62 years, suffering from severe gastric distress, pain, nausea, distension. There was an accompanying anorexia, anemia and extreme weakness of arms and legs. The cheilosis was not quite so typical as in the first case, because the fissures were not as prominent. They were smaller, and tended to bleed and later form encrustations. An inclination on the part of the patient to pick at these lesions resulted in some infection. The first dose of 100 mg B_6 hydrochloride was given intravenously. No treatment was given the next day. On the third day 50 mg B_6 hydrochloride was given by mouth and a second 50 mg on the fourth day. On the fifth day the lesions were entirely healed and did not return during the course of the illness, though the patient died two months later with a blood dyscrasia. The anorexia, anemia and severe weakness were unaffected.

The third case of cheilosis occurred in an 18-months-old white girl with a diagnosis of celiac disease. Her lesions were characterized by small fissures in the corners of the mouth with red areas surrounding them. They developed while she was subsisting on a diet composed primarily of milk and bananas. At this time the only change of regime was the intravenous administration of 50 mg of B_6 hydrochloride daily for 4 days. This resulted in the healing of the mouth lesions which remained healed during the rest of her stay in the hospital.

A fourth case of cheilosis, mild, but characteristic, occurred in a 27-year-old sprue patient. This patient was extremely deficient in many of the vitamin factors. There was slight improvement of the mouth lesion with B_6, but complete healing did not occur either with vitamin B_6 (1000 mg) or with a combination of B_6 (300 mg), riboflavin (140 mg) and nicotinic acid (1000 mg). The lesion finally healed completely, however, when 445 units of a concentrated liver extract‡ was given intramuscularly over a period of one week.

It is interesting in these 4 cases that the first was cured while receiving a basic diet deficient in riboflavin and the third developed the lesion while receiving what should have been an adequate supply of riboflavin from approximately one quart of milk a day and a fair amount of vitamin B_6 in the banana which was assayed in this laboratory and found curative of the typical B_6 deficient dermatitis of rats when fed in daily doses of 0.75 g.[8]

---

‡ Lederle solution liver extract parenteral refined and concentrated (N.N.R.).

We have found it true in controlled laboratory experiments that often a primary vitamin deficiency results in symptoms not altogether characteristic of that deficiency. This is brought about by the precipitation in the later stages of the primary deficiency of a secondary deficiency which is responsible for the symptoms observed.[9] It is probable that some such phenomenon may explain the curative powers of both riboflavin and B_6 in clinically similar lesions.

The nearest thing we have to an animal analogue of the cheilosis is the mouth lesion observed in the B_6-deficient rat. This occurs when as much as 100 μg of synthetic riboflavin are fed individually but yields to treatment with synthetic B_6. Similar lesions also occur in riboflavin-deficient rats but less frequently.

There are then 3 possible explanations of the observations that both riboflavin and B_6 cure cheilosis, (1) that riboflavin is the primary and specific deficiency responsible for the cheilosis and the B_6 operates only indirectly, (2) that vitamin B_6 is the primary and specific deficiency responsible for the cheilosis and the riboflavin acts only indirectly, and (3) that both riboflavin and vitamin B_6 are necessary to maintain the integrity of the lips at the mucocutaneous junction and that a deficiency of either will precipitate the lesion.¶

---

[8] Smith, S. G., unpublished data.

[9] Margolis, L. H., Margolis, G., and Smith, S. G., *J. Nut.*, 1939, **17**, 63.

¶ Since this paper went to press two more patients with cheilosis have been treated, one with vitamin B_6 (350 mg) and one with riboflavin (50 mg) and both responded promptly. It should be noted here that Aykroyd and Krishnam[10] have reported the cure of cheilosis with a yeast preparation freed from flavin.

[10] Aykroyd, W. R., and Krishnam, B. G., *Ind. J. Med. Res.*, 1938, **25**, 643.

11296

Sulfathiazole: Effect on *Staphylococcus aureus in vitro.*

Charles H. Rammelkamp and Chester S. Keefer.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

Recently several thiazole analogues of sulfapyridine have been described[1] and a few reports concerning their toxicity[2] and their protective action against *Staphylococcus aureus* in experimental animals[3, 4] have been made. It appears that the thiazole compounds may prove useful clinically. However, further information concerning their action both *in vitro* and in the experimental animal is desirable. It is for this reason that the present *in vitro* study with special emphasis on the action of sulfathiazole (2-sulfanilamidothiazole) on *S. aureus* was undertaken.

Methods. Of the 4 strains of staphylococci used, 3 were isolated from the blood stream of patients with bacteremia, and the other from a case of osteomyelitis of the femur. All 4 strains produced yellow pigment on agar, 2 produced hemolysis on blood agar, and all gave a strongly positive coagulase reaction.[5] The organisms were stored in the icebox on blood-agar slants, and 16-hour peptone-broth cultures were used. All dilutions were made in saline.

To study the comparative effects of sulfanilamide, sulfapyridine, sulfathiazole, and sulfamethylthiazole on staphylococci in whole blood the method employed previously by Spink and Keefer[6] was used. Defibrinated whole blood was obtained from individuals without signs of infection. To 0.5 cc of defibrinated blood 0.1 cc of various dilutions of the 16-hour broth culture was added. The chemicals were dissolved in saline and added in 0.1 cc amounts. The final concentration in each tube was 10 mg/100 cc unless otherwise stated. Tubes with 8 different dilutions of organisms were set up in duplicate, sealed in a gas-oxygen flame, and placed in a rotator in the incubator. The tubes were removed at 24 and 48 hours, and examined for hemolysis. Those tubes showing no hemolysis were then opened and the contents plated out and the colonies counted. The number of organisms present was calculated from the number contained in 2.5 cc of the sixth tube which was plated out at the beginning of the experiment.

---

[1] Fosbinder, R. F., and Walter, L. A., *J. Am. Chem. Soc.*, 1939, **61**, 2032.

[2] van Dyke, H. B., Greep, R. O., Rake, G., and McKee, C. M., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 410.

[3] Barlow, O. W., and Homburger, E., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 792.

[4] McKee, C. M., Rake, G., Greep, R. O., and van Dyke, H. B., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 417.

[5] Chapman, G. H., Berens, C., Peters, A., and Curcio, L., *J. Bact.*, 1934, **28**, 343.

[6] Spink, W. W., and Keefer, C. S., *J. Clin. Invest.*, 1936, **15**, 21.

Action in whole defibrinated blood. The majority of normal human defibrinated blood-samples could kill less than 10 staphylococci in 0.5 cc, although rarely as many as 1000 organisms were killed. When sulfathiazole was added to these samples the killing power was increased, and there was evidence of definite bacteriostasis in the tubes in which there was growth. To determine the concentration that produced the maximal effect, dilutions of sulfathiazole were made so that final concentrations of 1, –2.5, –5, –10, –15, and 20 mg/100 cc were obtained. Chart I demonstrates the bacteriostatic action of sulfathiazole in these various concentrations. The evidence of bacteriostasis as represented here was the absence of hemolysis at the end of 48 hours. Hemolysis in these experiments did not occur unless there were 10^8 organisms per cc. In those tubes

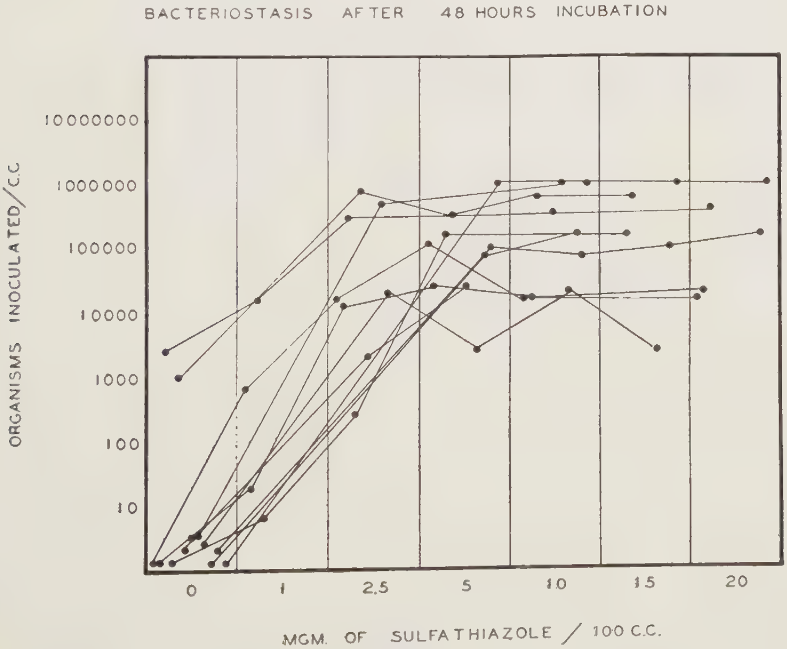


CHART 1.

Each dot represents the largest inoculum of *S. aureus* that failed to produce hemolysis after 48 hours' incubation. The lines connect individual experiments on the same sample of blood.

showing no hemolysis there were 10^4 organisms or less per cc. Similar results were obtained when the tubes were examined at the end of 24 hours, but the color change was not so marked. In the tubes containing the drugs, the hemolysis was greatly delayed as compared to the controls. From the chart it can be seen that the effective bacteriostatic level of sulfathiazole was between 2.5 and 5 mg/100 cc and that higher concentrations of the drug produced little increase in this action.

The increase in the bactericidal effect upon the addition of sulfathiazole was not as striking, but it was definite. Chart 2 represents a typical experiment on one sample of defibrinated blood. In all, 12 such experiments were done. Again, the results shown in this chart are taken after 48 hours' incubation in the rotator; however, the results at 24 hours were similar. Indeed, there was little change in the number of organisms between the 24th and the 48th hour. The

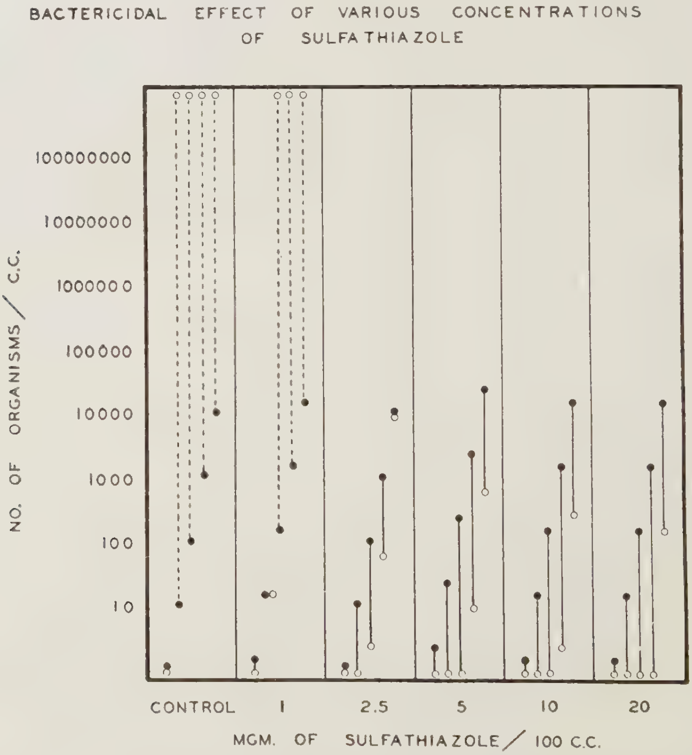


CHART 2.

Solid dots represent original inoculum per cc of *S. aureus*. Circles represent final number of organisms per cc at the end of 48 hours' incubation. Solid lines indicate killing; broken lines growth.

addition of sulfathiazole to defibrinated whole blood which kills less than 10 staphylococci increased the bactericidal power at least 100 times. In 2 experiments in which the blood killed 50 to 100 organisms the addition of sulfathiazole enhanced the bactericidal effect 10 to 100 times. Again it is evident from this chart that concentrations of between 2.5 and 5 mg/100 cc gave effective results, and that increasing the level above this concentration did not enhance the bactericidal effect appreciably.

The above observations were repeated after giving sulfathiazole by mouth to normal persons. Blood was withdrawn both before and after the drug was administered, and the organisms were added. The second sample showed an increase in both the bactericidal and bacteriostatic action against the staphylococcus as compared with the sample of blood before the drug was given.

Comparative effects of sulfanilamide derivatives. Since sulfanilamide and sulfapyridine have both been disappointing in their effects

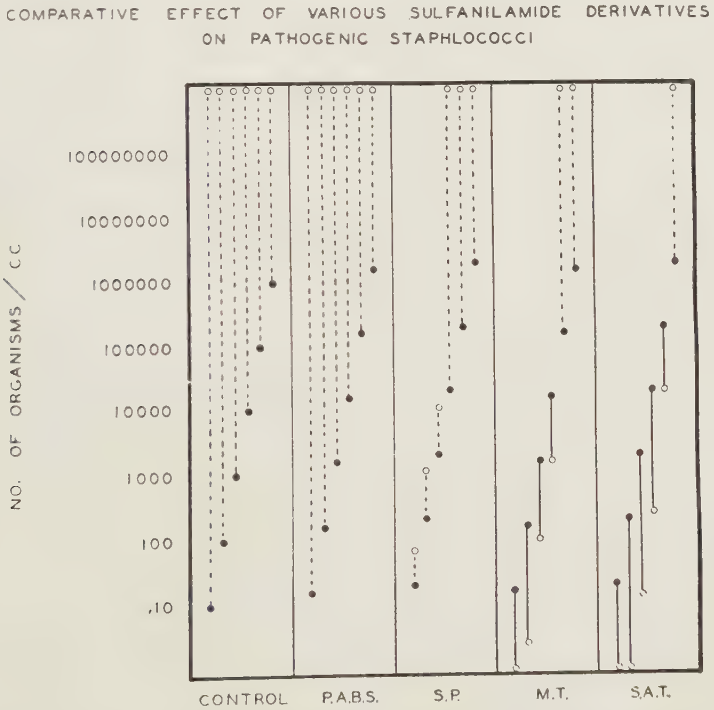


CHART 3.

Solid dots represent original inoculum. Circles represent number of organisms per cc at the end of 48 hours' incubation. Broken lines indicate growth; solid lines indicate killing. P.A.B.S. = sulfanilamide; S.P. = sulfapyridine; M.T. = sulfamethylthiazole; S.A.T. = sulfathiazole.

on the clinical course of cases with *S. aureus* bacteremia, it seemed desirable to compare the action of these drugs with the thiazole compounds (sulfathiazole and sulfamethylthiazole) *in vitro.*

The drugs were added to the blood so that the final concentrations were 10 mg/100 cc. In each of 8 experiments, the various drugs were added to the same sample of blood; 8 dilutions of organisms were made, and set up in duplicate tubes and incubated for 24 and 48 hours. The tubes were then opened and the contents cultured. Chart 3 illustrates the results of a typical experiment. When 10 or more organisms were added to the blood without any drug, and incubated for 48 hours, there were at least 10^8 organisms present at the end of 24 hours. With sulfanilamide the same was true. It was noted, however, that at the end of 24 hours there was evidence of bacteriostasis in that hemolysis of the blood was delayed. This, however, was never very striking. The bacteriostatic effect of sulfapyridine was shown when small numbers of organisms were used, that is less than 10,000 per cc of blood. Both sulfathiazole and sulfamethylthiazole showed bacteriostatic and bactericidal properties. The numbers of organisms actually decreased during incubation and this was striking even when as many as 100,000 organisms per cc were used. From our experiments, using whole blood, sulfathiazole was slightly superior to sulfamethylthiazole although the difference was not great. It seems justifiable to say that in whole defibrinated blood, sulfathiazole is more effective against staphylococci than sulfamethylthiazole, sulfapyridine, or sulfanilamide.

Conclusions. Sulfathiazole is an effective bacteriostatic and bactericidal agent against *S. aureus* when added to defibrinated whole blood *in vitro.* Concentrations between 2.5 and 5 mg/100 cc are necessary to obtain the maximal effect. Comparison of this drug with sulfamethylthiazole, sulfanilamide, and sulfapyridine show it to be somewhat superior in the experiments reported.

We wish to thank The Squibb Institute of Medical Research for the supply of sulfathiazole and The Department of Medical Research of the Winthrop Chemical Company for the supply of sulfamethylthiazole used in this investigation.

We wish to thank Miss Marjorie Jewell and Miss Eleanor Fleming for technical assistance.

11297

Effect of Insulin on Plasma Level and Excretion of Vitamin C.*

Elaine P. Ralli and Sol Sherry.

From the Department of Medicine, New York University College of Medicine.

In studying the metabolism of vitamin C in normal and depancreatized dogs, the urinary excretion of the vitamin was found to be strikingly reduced in the diabetic animal. The diets in both

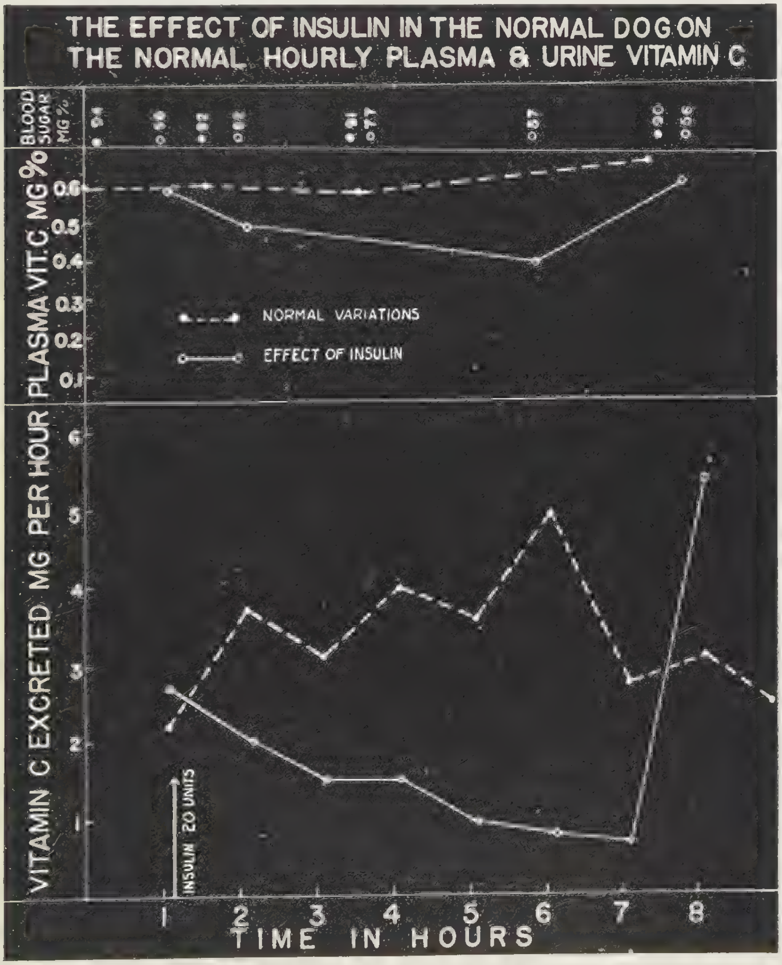


Chart 1.

---

* This research was aided by a grant from the Josiah Macy, Jr., Foundation.

groups of animals were totally devoid of vitamin C; dried meat powder was used in place of raw beef. The dogs were kept in individual metabolism cages and the urines were collected by catheter at the start and end of each 24-hour period. Urines voided between catheterizations were collected in dark bottles containing sulphuric acid and hydroxyquinoline to prevent the destruction of the urinary ascorbic acid. Fasting bloods were taken for the determination of the plasma vitamin C.[1] Urinary vitamin C was determined by the method of Evelyn.[2] The depancreatized dogs received insulin twice daily in amounts which allowed a moderate daily excretion of sugar.

The 6 normal dogs excreted an average of 20.6 mg of vitamin C per kg of body weight in 24 hours. The fasting plasma levels of vitamin C varied from 0.55 to 0.94 mg %. The 7 diabetic dogs maintained on insulin excreted on an average only 3.6 mg of vitamin C per kg of body weight in 24 hours. The fasting plasma levels varied from 0.33 to 0.71 mg %.

The influence of injected insulin on the plasma level and excretion of vitamin C was studied in a series of acute experiments in both the normal and depancreatized dog. Control experiments were run on each dog to ascertain the hourly plasma variation and urinary excretion of vitamin C. Over a period of 8 hours the plasma levels of vitamin C in the normal dog varied from 0.58 to 0.66 mg % and

TABLE I.
Average 24-hour Excretion of Vitamin C and Fasting Plasma Levels in Normal and Depancreatized Dogs on Insulin.

Dog No.	Wt, kg	No. daily observations	Urinary excretion vitamin C in mg daily		Urinary excretion per kg Body wt	Fasting plasma levels vitamin C mg %		
			Avg	S.D.		No. obser.	Avg	S.D.
207	15.75	13	304	±32.1	19.3	5	0.55	±.09
211	12.25	17	189	±20.6	15.4	8	0.65	±.07
197	11.25	10	232	±17.0	20.6	7	0.62	±.14
193	9.0	21	163	±18.3	18.1	13	0.63	±.08
215	11.75	1	300		25.5	1	0.89	
218	13.0	6	319		24.5	2	0.94	
			Diabetic Dogs.*					
192	9.75	25	14	± 6.5	1.4	9	0.45	±.03
214	6.75	5	8	± 1.6	1.2	2	0.41	
216	8.25	5	16	± 2.0	1.9	2	0.33	
170	12.50	15	71	±21.0	5.9	5	0.49	±.12
211	10.50	13	81	± 9.0	7.7	6	0.71	±.05
215	10.50	11	29	±11.0	2.8	5	0.66	±.12
208	11.50	5	52	±13.0	4.5	2	0.66	

*The depancreatized dogs received insulin twice daily.

---

[1] Mindlin, R. L., and Butler, A. M., *J. Biol. Chem.*, 1938, **122**, 673.

[2] Evelyn, K. A., Malloy, H. T., and Rosen, C., *J. Biol. Chem.*, 1938, **126**, 655.

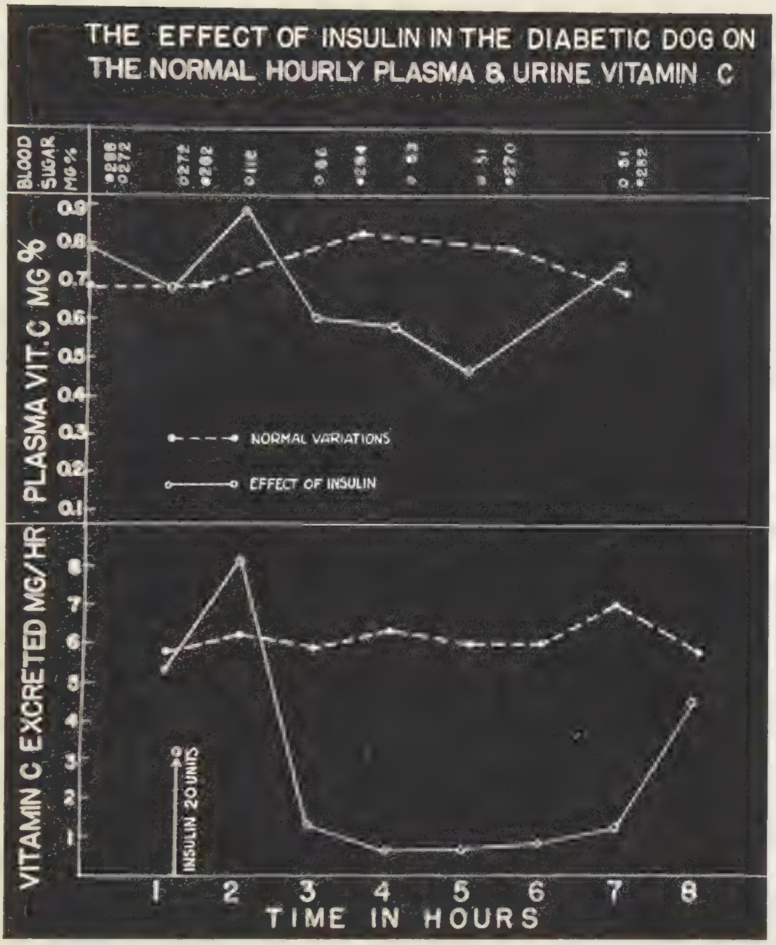


CHART 2.

the urinary excretion varied from 2.16 to 4.92 mg per hour (Chart 1). In the diabetic dog the plasma levels of vitamin C varied from 0.69 to 0.83 mg % and the urinary excretion from 5.70 to 7.04 mg per hour (Chart 2). In 3 experiments in the normal dog 20 units of insulin were injected subcutaneously and at the same time a small dose of glucose was given by mouth to avoid too rapid a fall in the blood sugar. One typical experiment is shown in Chart 1. In contrast to the normal variations in plasma levels of vitamin C, following the injection of insulin there is a fall in the plasma concentration and the urinary excretion of the vitamin. The effect lasted about 6-7 hours at which time both the plasma level and the urinary excretion returned to normal.

In the diabetic dog (Chart 2) in 3 experiments the results are similar. There was a sharp fall in the plasma level and urinary excretion of vitamin C as soon as the subcutaneously injected insulin became effective.

In additional experiments we have found that if glucose is administered intravenously at the time that the plasma vitamin C level and urinary excretion is reduced the effect can be overcome and the plasma level of the vitamin returned to normal.

Similar studies are being made at present on human normal and diabetic subjects.

11298 P

Test for the Sterility of Biologic Products.

M. S. MARSHALL, J. B. GUNNISON AND M. P. LUXEN.

From the Department of Bacteriology, University of California Medical School, San Francisco.

Standard tests for the sterility of finished vaccines or serums consist of the inoculation of 0.25 cc and 1.0 cc from the proper number of samples of the product to Smith fermentation-tubes containing infusion-broth. The tubes are heated within 5 hours before inoculation to drive off dissolved oxygen. They are then inoculated and are incubated for 7 days. The presence of weak points in the standard procedure has long been suspected.

The inherent weaknesses of the standard test are, in part, dependent upon (1) the frequent use of merthiolate which, because of its bacteriostatic effect, prevents the growth of contaminants, if any, in many instances; and (2) standard Smith tubes are not well adapted to the cultivation of anaërobes. The use of a second transfer, after 7 days, from optically clear Smith tubes to fresh Smith tubes in an endeavor to overcome the bacteriostatic action of the preservative is definitely irrational and thus unsatisfactory.[1]

The use of Brewer's medium[2] containing thioglycollate overcomes the objections given because it permits the growth of organisms in the presence of merthiolate and because it is suited to the cultivation of anaërobes. The results reported here support these views.

Tests were made with vaccines (bacterins) and serums, preserved with merthiolate, 1:5000 or 1:10,000, or with phenol, 0.5%. As contaminants 24-hour cultures of *Staph. aureus, Staph. albus, Ps. pyocyanea, B. subtilis* and *C. xerosis* were used.[3] In addition, there

---

[1] Marshall, M. S., and Hrenoff, A. K., *J. Infect. Dis.*, 1937, **61**, 42.

[2] Brewer, J. H., *J. Bact.*, 1940, **39**, 10.

TABLE I.
Growth from Contaminated Serum (1.0 and 0.25 cc Amounts).

Preserved with	In thioglycollate medium Contaminant		In infusion-broth (Smith tubes) Contaminant	
	Staph. aureus	*Cl. tetani*	*Staph. aureus*	*Cl. tetani*
Merthiolate, 1:5000	+	+	—	—
" 1:10,000	+	+	—	—
Phenol, 0.5%	+	+	+	—
No preservative	+	+	+	—

were available dried spores of *Cl. tetani* in sand, 10 years old, prepared by Coleman.[4] Vaccines and serums were inoculated lightly with these organisms, singly and in mixtures. From these products 0.25 cc and 1.0 cc respectively, containing from 5 to 500 organisms, were transferred to standard Smith tubes and also to tubes containing 12 cc of thioglycollate medium.* Incubation at 37°C for 7 days followed, with frequent observations.

A summary of results is given in Table I. No difference between results with vaccines and serums was noted. A hundred-fold increase in the inocula did not change the results. *Ps. pyocyanea* appeared to be less sensitive to merthiolate than other organisms; serum preserved with merthiolate, 1:10,000, usually yielded growth and, after several days, serum with merthiolate, 1:5000, usually yielded growth, even in ordinary broth. Dried spores of the *Cl. tetani* failed to develop in Smith tubes in pure culture, although they grew quickly in thioglycollate medium. They failed to develop in Smith tubes in pure culture even from unpreserved serum or serum preserved with phenol. Numerically, an inoculum of 150 spores, the maximum tested, failed to grow in Smith tubes; 10 spores, the lowest number tested, grew readily in thioglycollate medium. In fact, growth of these spores in Smith tubes was observed only when aërobes were also present, and this was irregular.

In summary, it seems clearly evident that the standard test for sterility is inadequate because of the inhibition of growth of possible contaminants by merthiolate, or similar bacteriostatic substances, and because the method is not reliable for anaërobic contaminants. It seems equally clear that thioglycollate medium is adapted to meet these objections and, with suitable refinements, should replace the standard test for sterility.

---

[3] Rosenstein, C., Levin, I., and Levin, H., *Am. J. Hyg.*, 1935, **21**, 260.

[4] Coleman, G. E., *J. Infect. Dis.*, 1930, **47**, 410.

* Furnished by the Baltimore Biological Laboratories. The formula is: pork infusion from 37.5 g per liter; thiopeptone, 1%; dextrose, 1%; NaCl, 0.5%; Na thioglycollate, 0.1%; agar, 0.05%; methylene blue, 0.0002%.

11299 P

Presence of a Principle with Progesterone-like Activity Obtained from a Plant Source.

G. de Sütö-Nagy.* (Introduced by C. N. H. Long.)

From the Institute of General Pathology, University of Budapest.

The known biological incompleteness of the protein fraction of the bean has received considerable attention in interpretations of the malnutrition caused by a raw bean diet. Belák and Szathmáry,[1, 2] and Zselyonka and Illényi[3] were the first to point out that, together with this loss of weight, there was also observable an inhibition of the estrus of mice during the period of feeding with a diet containing 15% of raw bean. They isolated a globulin fraction from the bean flour, which they referred to as "phaseolin." Together with a loss of weight, the inhibition of the estrus of the mice could be observed when 5% of the basic food of the mice was replaced by this protein fraction. The fat-soluble fraction of the bean was found by the same authors to be ineffective. Selye and Collip,[4] in replacing 30 to 50% of the basic food of the rat with bean flour, observed a certain atrophy of the ovaries and interpreted their findings as a consequence of a "malnutrition."

In our feeding experiments, first using rats as test animals, and following the course of the estrous cycles by means of the vaginal smear method of Long and Evans,[5] we were able to establish the following:

1. Complete inhibition of the normal estrous cycle could be observed when 15% of the basic food was replaced by bean flour. Controls, on a diet without bean flour, failed to show any change in their regular cycles, whether that diet was offered to them (a) *ad libitum,* or (b) in an amount 15% less by weight than that regularly taken by these animals.

2. The pure globulin fraction of the bean, as prepared by Osborne,[6] given in 5% by weight to the basic food did not inhibit the

---

* Present address, Department of Chemistry, Yale University, New Haven, Conn.

[1] Belák, S., and Szathmáry, J., 5th Congress of the Hungarian Physiol. Soc., Decbrecen, June, 1935.

[2] Belák, S., and Szathmáry, J., *Biochem. Z.*, 1937, **291**, 259.

[3] Zselyonka, L., and Illényi, A., *Biochem. Z.*, 1937, **291**, 263.

[4] Selye, H., and Collip, J. B., *Endocrinology*, 1936, **20**, 667.

[5] Long, J. A., and Evans, H., *Memoirs of the Univ. of California*, 1922, **6**.

estrus of the rats. The "phaseolin", however, as prepared by Zselyonka and Illényi,[3] inhibited the estrus when added at the same level to the basic food. Osborne's method differs substantially from the other in that it involves a thorough extraction of the bean proteins with lipoid solvents.

3. The raw bean flour, mixed in the diet to 15% inhibited the estrus of rats. However, the same bean flour was found ineffective in this sense even at a 20% level, after its thorough extraction in a Soxhelet apparatus, or after repeated thorough extraction at room temperature with fat solvents.

4. Furthermore, a crude oil extracted from the bean flour with fat solvents at room temperature in about 1.7% yield inhibited the estrus of the rats during the period of its subcutaneous administration. Controls, receiving the same amount of olive oil continued to show their regular estrus.

5. It was found that this estrus-inhibiting principle in the oil of the white bean, as extracted with lipoid solvents, is directly antagonistic in its action on castrated female rats to the estrogenic hormone. One gamma estrogenic hormone (international standard ketohydroxyestrin), injected subcutaneously, first proved to be sufficient in our castrated rats to evoke estrus in about 100%. In cross-experiments the same amount of estrogen was found to be 50% less effective when the test groups were kept on a 15% by weight bean diet prior to, during, and after the administration of the estrogenic hormone.

The results of these experiments seemed to indicate that in the lipoid-soluble fraction of the bean there is present a factor with a biological action similar to that of progesterone.

6. In order to examine this point further, the crude bean oil was tested in the Clauberg test, known as a most specific one for progesterone-like effects. We used the abbreviated form of the Clauberg test, as proposed by Butenandt, *et al.*[7]

With this test a positive progesterone-like effect of the bean oil could be repeatedly demonstrated. This is the first example of a substance from a plant source which exerts a progesterone-like effect.

This progesterone-like principle of the bean was found to be very sensitive to heat, oxidative agents and alkalies. Only extracts which took account of these properties retained their potency.

---

[6] Osborne, T. B., and Strauss, E., *Abderhalden's Handbuch d. biol. Arbeitsmethoden*, Abt. I, Bd. 8, p. 425.

[7] Butenandt, A., Westphal, U., and Holweg, W., *Z. physiol. Chem.*, 1934, **227**, 84.

11300 P

Quantitative Studies on Antithrombin.*

SLOAN J. WILSON. (Introduced by E. von Haam.)
From the Department of Pathology, Ohio State University, Columbus, Ohio.

The normally existing anticoagulants of blood serum and plasma according to present concepts are antiprothrombin and antithrombin. Howell[1] stated that blood normally contains but a small amount of antithrombin. A critical analysis of previous methods for the quantitative determination of antithrombin has only become possible with the purification and standardization of prothrombin and thrombin by Warner, Brinkhous, Smith and Seegers.[2] Previous methods have consisted of either adding considerably less than one unit of thrombin to undiluted serum and observing the clotting time upon the subsequent addition of fibrinogen after various periods of incubation[3, 4, 5] or by adding a small fraction or a few units of thrombin to plasma and observing the variations in the clotting time.[6, 7] The obvious conclusion was that serum or plasma can inactivate or neutralize very little thrombin. It is well established that hemorrhage may occur with plasma prothrombin levels of 35% of normal (105 units) or less. Theoretically there should be a marked excess of prothrombin even in this hemorrhagic zone inasmuch as one unit of prothrombin when converted to thrombin will convert 1 cc of fibrinogen to fibrin in 15 seconds. From these observations it would seem probable that the antithrombic activity of serum and plasma is much greater than previously assumed.

Before a method was devised for the quantitation of antithrombic activity of serum or plasma, the quantitative adsorption of thrombin was observed during the conversion of fibrinogen to fibrin. To various amounts of purified fibrinogen were added 60 units of

---

* Aided by a grant from the Comly Research Fund, Ohio State University.

[1] Howell, W. H., *Physiol. Rev.*, 1935, **15**, 435.

[2] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667; Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801; Seegers, W. H., Brinkhous, K. M., Smith, H. P., and Warner, E. D., *J. Biol. Chem.*, 1938, **126**, 91.

[3] Howell, W. H., *Arch. Int. Med.*, 1914, **13**, 76.

[4] Gasser, H. S., *Am. J. Physiol.*, 1916, **42**, 378.

[5] Mills, C. A., and Kitzmiller, K. V., *Arch. Int. Med.*, 1926, **38**, 544; *ibid.*, 1927, **39**, 618.

[6] Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

[7] Eagle, H., *Bull. Johns Hopkins Hosp.*, 1937, **60**, 428.

purified thrombin and the amount that disappeared from solution calculated. The conversion of 1 mg of fibrinogen resulted in the adsorption of 5.1 units of thrombin upon the fibrin strands; no further adsorption occurred regardless of the time of incubation in the presence of excess amounts of thrombin. Therefore when blood coagulates, approximately 90% of the thrombin must be inactivated or neutralized by the normal antithrombic activity of the serum.

Observations were then made on the quantitative activity of the antithrombin of diluted and undiluted serum and oxalated plasma. The fibrinogen was removed from plasma by incubation for 10 minutes at 56°C. When 1 unit of thrombin was added to 1 cc of various dilutions of serum and plasma and the amount of thrombin calculated that was inactivated at various periods of incubation at 28°C, it was observed that there was a quantitative correlation in antithrombic activity at 4 minutes' incubation in dilutions greater than one part in forty-five. There was little or no difference in the antithrombin of serum and plasma. In the ultimate test the incubated (56°C, 10 minutes) serum or plasma was diluted (usual dilutions, 1 part in 40, 50, or 60) to such an extent that 1 cc would inactivate 0.48 to 0.59 units of thrombin in 4 minutes at 28°C. In such dilutions no correction was necessary for the thrombin-neutralizing activity of oxalates. However, when the results of such a test were compared with the ability of undiluted serum or plasma to neutralize or inactivate thrombin the calculated result had to be multiplied by a correction factor of 5.28. The addition of small amounts of thrombin to undiluted serum or plasma was no index of the antithrombic activity because of the unexplained phenomenon that small fractions of a unit of thrombin may remain active for considerable time. One unit of antithrombin is defined as that amount which will neutralize or inactivate 1 unit of thrombin in 4 minutes incubated at 28°C.

TABLE I.
Normal Levels of Antithrombin in Human Subjects and Various Animals.

Source of blood	No. of subjects	Avg antithrombin units per cc	Range of variation units per cc*
Human, normal	34	90	74-115
Dog	15	87	74-105
Cat	5	96	79-117
Cow	11	95	82-119
Pig	4	103	101-109
Guinea pig	10	108	97-120
Rabbit	18	108	98-133
Rat (albino)	28	123	102-147

*Lowest and highest determinations.

The antithrombin of the serum and plasma of various species was then determined (Table I). Human serum and plasma contain between 74 and 115 units per cubic centimeter. In human subjects with the plasma prothrombin within the hemorrhagic zone the prothrombic unitage usually approximated or was lower than the antithrombic unitage.

Summary. A method is devised for the quantitative determination of antithrombic activity of serum and plasma. One unit of antithrombin is defined as that amount which will inactivate or neutralize 1 unit of thrombin in 4 minutes' incubation at 28°C. There is little or no quantitative difference in the antithrombin of serum and plasma. The antithrombic activity of normal serum and plasma is considerably greater than has been previously described.

11301 P

Diphtheria-Antitoxin Production After Intravenous or Subcutaneous Injection of Alum-Toxoid.

JULES FREUND AND MARY V. BONANTO.

From the Department of Health, City of New York, Bureau of Laboratories, Antitoxin Laboratory, Otisville, N. Y.

The purpose of the present study is to compare the effectiveness of intravenous and subcutaneous injections of alum-precipitated diphtheric toxoid in stimulating antitoxin-formation in the rabbit. The general opinion that the subcutaneous is superior to the intravenous route in antitoxin-production is based on experiments with horses[1] and guinea pigs.[2] Further important difference between the previous and the present experiments is that in the previous studies the antigen, toxin or toxoid, was used in solution and in the present study the toxoid was employed as particulate material.

When bacterial suspensions are employed as antigen the vascular route is the more effective one. This is true not only in regard to pneumococci that rapidly become gram-negative when introduced into the skin,[3] but also in regard to heat-killed tubercle bacilli that are quite resistant.[4] The question can be raised whether toxins or toxoids would behave like bacteria, if they are injected as particulate material. By the subcutaneous route toxins and toxoids in combination with particulate material or as precipitates were employed by Ramon[5] and Glenny and his associates.[6]

---

[1] Kolle, W., and Wassermann, A., *Handbuch der path. Mikroörganismen,* **1912**, Verlag Gustav Fischer, Jena.

[2] Neill, J. M., Sugg, J. Y., and Richardson, L. V., *J. Immunol.*, 1935, **28**, 363.

[3] Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 441, 449; Dubos, R. J., and MacLeod, C. M., *J. Exp. Med.*, 1938, **67**, 269.

Four rabbits received intravenous and 5 rabbits subcutaneous injections of 1 cc of alum-precipitated diphtheric toxoid containing 25 L_f on 4 successive days and after a rest of 3 days a similar course of 4 injections. The antigen was diluted 1:10 with salt solution for the intravenous injections. The animals were tested 12, 18, and 25 days after the first course of 8 injections. Thirty-two days after the first course of injections the rabbits were given a new series of injections, but this time 5 days apart. The rabbits were bled approximately 5 days after each injection. The titrations were carried out by the intracutaneous method.

The results are presented in Table I. The first 3 samples of blood taken at different intervals after the first series of 8 injections show that the rate of antitoxin-formation was strikingly higher in the group with intravenous injections, since at the time of the first bleeding the average level in this group was 1.8 and in the group with subcutaneous injections only 0.1 antitoxin unit per cc. At the

TABLE I.
Antitoxin Units per cc of Serum in Rabbits Immunized with Alum-precipitated Diphtheric Toxoid.

		Days after the first course of injections			During the second course of injections. After						
Route	Rabbit No.	12	18	25	1st	2d	3d	4th	5th	6th	7th
Intravenous	1	5.0	4.0	2.0	15.0	15.0	20.0	15.0	12.5	12.5	10.0
	2	1.0	1.0	2.0	11.0	15.0	20.0	—	18.0	18.0	15.0
	3	1.0	2.0	1.0	11.0	12.0	15.0	15.0	10.0	10.0	7.5
	4	1.0	1.0	0.5	3.0	7.0	7.5	—	7.5	7.5	5.0
Avg		*1.8*	*2.0*	*1.3*	*10.0*	*12.2*	*15.6*	**16.0*	*12.0*	*12.0*	*9.3*
Subcutaneous	5	.1	2.0	.5	3.0	7.0	5.0	5.0	3.0	2.5	2.5
	6	.1	1.0	.5	3.0	6.0	5.0	2.5	2.0	3.0	2.5
	7	.1	2.0	.5	3.0	5.0	4.0	2.5	3.0	2.5	2.5
	8	.1	1.0	.5	2.0	5.0	4.0	2.5	3.0	2.5	2.5
	9	.1	0.1	.1	1.0	3.0	3.0	2.5	2.0	3.0	2.5
Avg		*.1*	*1.2*	*.4*	*2.4*	*5.2*	*4.2*	*2.5*	*2.7*	*2.8*	*2.5*

*Pooled sera.

---

[4] Freund, J., and Opie, E. L., *J. Exp. Med.*, 1938, **68**, 273.

[5] Ramon, G., *Revue d'Immunol.*, 1939, No. 5, 385.

[6] Glenny, A. T., Pope, C. G., Waddington, H., and Wallace, U., *J. Path. and Bact.*, 1926, **29**, 38.

time of the second and third bleedings the level was only slightly higher in the animals with intravenous injections. The 2 injections of the antigen that followed the rest-period stimulated a sharp rise in the titers in both groups while the following injections produced a rise only in the group with intravenous injections. At the time of all of the 7 bleedings during the second course of injections the antitoxin levels were conspicuously higher in the group that received intravenous injections.

Conclusion. Antitoxin-formation in the rabbit is more rapid and abundant after intravenous than after subcutaneous injections of 25 L_f alum-precipitated diphtheric toxoid.

11302

Experiments on Cultivation of Virus of Infectious Avian Encephalomyelitis.*

I. J. Kligler and P. K. Olitsky.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

In connection with investigations on the virus of infectious avian encephalomyelitis (A.E.),[1] we sought to enhance antigen quantitatively for the preparation of immunizing vaccines, by cultivating the virus in developing chick embryos and in their tissues *in vitro*. The results relating to epidemiology and to additional properties of this newly discovered virus were considered of sufficient importance to be offered in the present paper.

Materials and Methods. The strain of virus employed was that kindly sent to us by Dr. Van Roekel and which was described by him[1] as well as by Olitsky.[1] The procedures followed closely those which had been employed by the latter. Ten percent infected-chick-brain suspension in broth was used to initiate the various cultures. Tests for virus, except as noted otherwise, were made by intracerebral inoculation of 0.05 to 0.1 cc of tenfold dilutions of the material to be examined into 2- or 3-weeks-old Rhode Island, or New Hampshire, Red chicks.

---

* We express our debt to Mr. P. Haselbauer for his invaluable aid.

[1] Olitsky, P. K., *J. Exp. Med.*, 1939, **70**, 565; Olitsky, P. K., and Bauer, J. H., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 634. For earlier descriptions, see: Jones, E. E., *J. Exp. Med.*, 1934, **59**, 781, and Van Roekel, H., Bullis, K. L., and Clarke, M. K., *J. Am. Vet. Med. Assn.*, 1938, N.S. **46**, 372.

Fertile eggs of White Leghorn chickens were available. It was shown that of 11 normal, 2-week-old birds of this breed given 0.05 cc of 10^{-1} dilution of active brain intracerebrally, all developed characteristic encephalomyelitis after 11 to 16 days. Hence the Leghorn chicks are susceptible to the A.E. virus.

Cultivation Experiments. 1. *In Developing Chick Embryos.* In view of the fact that the virus is active only in avian hosts and that it has been reported[2] that the disease can be transmitted by way of the egg, the first attempts at cultivation were made in embryonated eggs as the most promising approach to the problem. Since the virus was shown to be active in nervous tissue, it was thought desirable to employ embryos of eggs of 5 or 6 days' incubation, which had been used successfully for the cultivation of rabies-virus.[3]

Parallel cultures were set up, employing 1,000 chick cerebral-test-doses of A. E. virus, one group being inoculated by way of the yolk-sac, after the manner of Cox;[4] another, through an opening over the embryo, directly into the allantois. The eggs were then incubated at 36.5°C for 6-10 days, when their contents were removed and passage made from the allantoic membrane, yolk-sac, embryonic tissue, and brain to new series of eggs. The different tissues of the 2d and 4th passages were ground in broth and decimal dilutions of 10^{-2} to 10^{-5} or 10^{-6} were injected intracerebrally into chicks. Five series of cultures were made and tested in 175 chicks, and in no instance could viral multiplication be discerned. In 2 instances, the brains of chicks hatched from such inoculated embryos were passaged in young chicks with negative results. Pathological examination of the CNS of embryos 18-20 days old (*i.e.,* 12-14 days after inoculation), or of hatched chicks failed to reveal the characteristic lesions of encephalomyelitis.

In addition, tests were made on the survival of virus injected into young and older embryos. In the former, 21 eggs with 5- to 7-day-old embryos, and in the latter, 11 eggs with 11- to 13-day-old ones were used; 0.1 cc of 10^{-1} dilution of virus (active in 10^{-5} dilution) was inoculated into the eggs through the allantois in the region of the embryo. The presence of virus in the embryos was demonstrated by pooling 2 or 3 inoculated embryos 1 hour later and injecting decimal dilutions of the suspension into chicks. After incubation at 37°C for varying periods, the embryonic brain and the other tissues were again examined for virus-content. In the series of young embryos, virus was recovered in dilutions up to 10^{-3} after 24, 48, and 72 hours. but not at all after 5 or 9 days; in the older ones, virus was recovered in a dilution of 10^{-4} after 48 hours, but not after 8 and 11 days. Pathological examination of the brain and cord of these embryos 11 days after inoculation failed to show any characteristic lesions.

---

[2] Van Roekel, H., Bullis, K. L., and Clarke, M. K., *Vet. Med.*, 1939, **34**, No. 12.

[3] Kligler, I. J., and Bernkopf, H., *Nature,* 1939, **143**, 899.

[4] Cox, H. R., *Pub. Health Rep.*, 1938, **53**, 2241.

Finally, 21 of the birds which had hatched out of inoculated eggs or had received intracerebral inoculation of the mentioned embryonic material, and failed to show signs of the malady for at least 40 days, were then subjected to an intracerebral test for immunity. All came down with characteristic A. E. virus infection, thus indicating the absence of active multiplying virus in the chicks and in the materials used in an amount sufficient to induce immunity.[1] At the time of the test, the fowls were 9-10 weeks of age.

2. *In Minced Embryonic Tissues in vitro.* Three series of cultures of this type were set up. In one, minced chick-embryo brain plus Tyrode's solution and 10% chicken serum served as medium. The second series was the same as the first, except that minced whole chick embryo was used in place of brain. In the last, Rivers' medium was used: minced chick embryo in Tyrode's solution. In each instance the viral suspension, as well as 2 flasks of the inoculated culture, was titrated for determination of the viral titer at the outset. (Similar titrations of 2 or 3 culture flasks were carried out at the time of transfer.) The cultures were incubated at 36.5 to 37°C and transfers into the respective media were made at 5-6 days' intervals. Each series was carried through 5 passages, so as to exclude any possible dilution factor of the original viral inoculum. The results are summarized in Table I and reveal that in the culture of whole-embryo tissue plus serum, there was a viral titer of 10^{-3} at the outset and at least 10^{-2} in the 5th subplant, although the original virus itself was calculated as being diluted to 10^{-8} in this passage. In the other 2 series the virus was detectable only through the 2d subplant; this corresponds to the point beyond which the virus is diluted out of its original activity.

Again it is clear that the avian virus has distinctive requirements for multiplication, such as are not found to exist for several others. Multiplication took place in minced whole embryos suspended in serum-Tyrode-solution mixture. The brain cultures were negative after the 2d subplant, as were those made with minced embryonic tissue in Tyrode's solution without serum.

It would appear, therefore, that the virus is rapidly lost at 37°C unless embryonic tissue and serum are present.

TABLE I.
Viral Activity in Various Minced Chick Embryo Media.

Titer of stock virus used for seeding cultures	Media	Titer of virus + media immediately after set-up	Subplants				
			1st	2d	3d	4th	5th
10^{-5}	Brain + serum	10^{-3}	10^{-3}	$<10^{-2}$	0	0	0
10^{-5}	Embryo + serum	10^{-3}	$>10^{-4}$	10^{-2}	10^{-4}	10^{-4}	10^{-2}
—	Embryo + Tyrode's solution	10^{-4}	$>10^{-2}$	10^{-2}	0	0	0

Summary and Discussion. 1. Embryonic chicks are apparently not susceptible to infection with this avian virus, although birds just hatched are. This is a strikingly reversed state from that which prevails in the case of certain other viruses,[5] which multiply readily in the undifferentiated tissues of the developing embryo but are inactive in the hatched chick. Whatever the reason may be, a fact of epidemiological significance emerges: the disease-agent is probably not transmissible by way of the egg, thus supporting the prior finding of Jones.[1]

2. The virus multiplied in minced whole-embryo tissue-cultures *in vitro* only under certain indicated conditions. The method in its present state, however, is not favorable for obtaining large yields of highly potent virus for use in immunizing procedures. No multiplication of virus was noted in this medium when chick-embryo brain was used instead of whole-embryo tissue. It is of interest in this connection that mammalian embryo-brain cultures have been found suitable for the multiplication of the neurotropic viruses of poliomyelitis[6] and of rabies.[7]

---

[5] For a discussion and references, see: Rivers, T. M., and Schwentker, F. F., *J. Exp. Med.*, 1932, **55**, 911; and Mackenzie, R. D., *J. Path. and Bact.*, 1933, **37**, 75.

[6] Sabin, A. B., and Olitsky, P. K., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 357.

[7] Kanazawa, K., *Japanese J. Exp. Med.*, 1936, **14**, 519; Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1937, **66**, 125; Bernkopf, H., and Kligler, I. J., *Brit. J. Exp. Path.*, 1937, **18**, 481.

11303

"Spreading" Properties and Mucolytic Activity of Leech Extracts.

ALBERT CLAUDE.

From the Rockefeller Institute for Medical Research, New York City.

In addition to their well known anticoagulating properties, leech extracts have the power to increase skin permeability.[1] Chemical studies from this laboratory have shown that the so-called spreading or "Reynals" factors, either from testicle,[2] or from the leech, were proteins.[3, 1] Recently, Chain and Duthrie[4] found that testicular extract would destroy the viscosity of the synovial fluid, and they have shown that this effect was due to the action of an enzyme on a polysaccharide. These authors suggested that this "mucinase" and the testicular spreading factor might be identical. If this is correct, one may expect leech extracts to have a high mucolytic activity, since their spreading power is 50 to 100 times greater than that of testicular extracts. In preliminary tests it was found that leech extract would destroy the viscosity of chicken tumor I extracts[5] with a remarkable speed, even at room temperature.

In the following experiments, the spreading power of various leech extracts was compared with their mucolytic activity, as measured by their action on chicken tumor I mucin.

Effect of Leech Extracts on Chicken Tumor I mucin. Previous work has shown that the spreading factor is located in the anterior part of the leech (head).[1] It is assumed that the substance is produced by the pharyngeal epithelium and plays a rôle in the process of feeding. On the other hand, a similar extract from the rest of the body is found to contain very little of the same factor.

In the present experiments, separated heads and bodies were extracted, in the usual way,[1] with 10 to 20 times their own weight of distilled water or phosphate buffer at pH 7.0, and centrifuged at 18,000 times gravity for 2 hours. These extracts were used as the source of enzyme. The substrates for mucolytic activity were concentrated extracts of chicken tumor I desiccates,[5] purified by high speed centrifugation. The speed of the enzymatic reaction was studied at a temperature of 25°C.

---

[1] Claude, A., *J. Exp. Med.*, 1937, **66**, 353.

[2] Duran-Reynals, F., *J. Exp. Med.*, 1929, **50**, 327; Hoffman, D. C., and Duran-Reynals, F., *Science*, 1930, **72**, 508; *J. Exp. Med.*, 1931, **53**, 387; McClean, D.. *J. Path. and Bact.*, 1930, **33**, 1045.

[3] Claude, A., and Duran-Reynals, F., *J. Exp. Med.*, 1937, **65**, 661.

[4] Chain, E., and Duthrie, E. S., *Nature*, 1939, **144**, 977.

[5] Claude, A., *J. Exp. Med.*, 1935, **61**, 27.

Under these conditions the leech head extract, in the concentration of 1%, by volume, was found to have a powerful effect on the chicken tumor mucin, bringing the relative viscosity of the extract from 7.7 to 1.5 in 5 to 10 minutes. At that concentration, 30 minutes of contact with the leech factor brought the viscosity of the fluid close to that of water, the reduction in viscosity corresponding to 99.25% of the original value. Leech head extract, in a final concentration of 0.01% reduced the original viscosity by 73.6% whereas the leech body extract, in a final concentration of 1%, brought about a reduction in viscosity of 73.9%. This indicates that a 0.01% head extract and a 1% body extract have about equal strength, the former being then about 100 times more active than the latter.

The spreading power of the leech extracts was tested by the injection of 10-fold dilutions of the solutions, mixed with India ink, in the rabbit skin.[2, 1] The head extract, at a dilution of 10^{-3}, gave an area of spread equal to 20.3 sq cm, as compared with 22.6 sq cm for the body extract, at 10^{-1} dilution. This would indicate that the head extract is about 100 times more active than the body extract. The above results are illustrated in Table I.

The close parallelism between the rate of action of the mucinase and the spreading power of different extracts is strong evidence that the two factors, in the leech, are identical.*

This view is also supported by the fact that bull testicular extract was about 100 times less active than the leech extract, as regard both spreading power and mucolytic activity.

Mechanism of Spread. No satisfactory explanation has been found to account for the phenomenon of spread.[1] The spread, considered as the result of the mucolytic activity of the spreading factor would assume the presence, in the skin, of a chemically suitable substrate for the mucinase to act upon. From the histochemical studies of Bensley[6] and Sylvén[7] it appears that normal or pathological tissues contain a viscid ground substance which, from its staining properties, resembles mucin.

In the present work freshly removed rabbit skin was passed through a meat grinder and extracted by contact with 2 volumes of a 10% NaCl solution at 2°C for 24 hours. This extract was filtered

---

* The present work has been confined to the study of changes in viscosity under the effect of leech extracts assuming that the spreading phenomenon is brought about by similar changes in the skin without requiring necessarily complete hydrolysis of the substrate.

[6] Bensley, S. H., *Anat. Rec.*, 1934, **60**, 93.

[7] Sylvén, B., *Virchows Arch. Path. Anat.*, 1938, **303**, 280.

TABLE I.
"Spreading" Property and Mucolytic Activity of Leech Extracts.

Leech head extract					Leech body extract				
Spread in rabbit skin			Mucolytic activity		Spread in rabbit skin			Mucolytic activity	
Amt solids injected, g	Area of spread, cm^2	Ratio active spread to spread of control	Conc. leech extr. in mucin sol. (by vol.), %	Reduction viscosity after 30 min. at 25°C, %	Amt solids injected, g	Area of spread, cm^2	Ratio active spread to spread of control	Conc. leech extr. in mucin sol. (by vol.), %	Reduction viscosity after 30 min. at 25°C, %
3.6×10^{-3}	91.0	16.0	1.00	99.3	2.35×10^{-3}	66.9	11.7	1.0	73.9
3.6×10^{-4}	38.0	6.6	0.10	97.9	2.35×10^{-4}	22.6	4.0	0.1	35.2
3.6×10^{-5}	31.0	5.4	0.01	73.6	2.35×10^{-5}	8.0	1.4		
3.6×10^{-6}	20.3	3.2			2.35×10^{-6}	6.3	1.0		
3.6×10^{-7}	6.8	1.2			2.35×10^{-7}	7.0	1.0		
3.6×10^{-8}	8.0	1.4			2.35×10^{-8}	7.5	1.3		
3.6×10^{-9}	5.5	1.0			2.35×10^{-9}	6.2	1.0		

TABLE II.
Mucolytic Activity of Leech Extract on Normal Rabbit Skin Mucoprotein.

Tests	Mucoprotein solution		Leech extract (head) Amt added to mucoprotein solution		Mucolytic activity			
					30 min at 25°C		30 hr at 25°C	
	Solids in sol. mg per cc	Relative viscosity of sol.	By volume, %	Final conc. in mixture, %	Relative viscosity of mixtures	Reduction in viscosity %	Relative viscosity of mixtures	Reduction in viscosity %
I	0.28	1.44	0.74	0.003	1.12	72.7		
II	0.70	2.2	1.0	0.004	1.38	68.4		
,,	0.70	2.2	0.1	0.0004	1.43	64.0		
III	0.70	2.2	1.0	0.004	1.46	61.7	1.32	73.4
IV	1.40	3.46	1.0	0.004	2.05	57.3	1.80	67.5

through paper and the filtrate treated with chloroform, with occasional shaking, for another 24 hours. Denatured proteins were discarded by centrifugation. The clear solution was then dialyzed in cellophane bags until free of NaCl. A protein fraction, different from the mucoprotein, separated out on dialysis, and was discarded by filtration through paper. All the operations were conducted in the cold, to avoid the action of a mucolytic enzyme which might have been extracted from the skin, together with the mucoprotein.

The skin mucoprotein is readily soluble in water, giving clear, slightly yellow solutions. The biuret reaction is positive. Solutions containing 0.7 mg solids per cc gave a strongly positive orcinol test. Like chicken tumor I mucin, it gives a stringy precipitate with neutral red. The behavior of the substance in acidic solution is noteworthy. Its solubility decreases progressively down to pH 4.0, giving a flocculent precipitate. At pH 4.0 there is a sudden change in the appearance of the precipitate which is then mucoid, and contracts. Below pH 4.0 the protein becomes more soluble but the precipitate retains its mucoid character. Below pH 2.0 the substance is completely soluble. On account of the physical change taking place at that point, it is uncertain whether pH 4.0 is the point of minimum solubility of the protein.[†]

A solution containing 1.4 mg skin mucoprotein per cc had a relative viscosity of 3.46 at 25°C. Addition of leech extract to a final concentration of 0.004% produced a sudden drop in the viscosity of the solution. As a result of 5 different tests, it appears that leech extracts, at the above concentration, and acting for 30 minutes at 25°C, will reduce the original viscosity of the solution by 68.4%. A contact of 30 hours at the same temperature will show but a slight additional effect, the total reduction in viscosity amounting to 70.4% of the original value. These results are in agreement with the observations of Meyer and coworkers,[8] who showed that only 69% of the synovial mucin was hydrolyzed by 45 hours' incubation with a pneumococcus enzyme, in contrast with 96% hydrolysis for the free polysaccharide, under the same conditions.

Summary and Conclusions. 1. Leech extracts contain a powerful mucolytic enzyme, as shown by its effect on the viscosity of chicken tumor I extracts. 2. Leech head extracts exhibit a mucolytic activity considerably greater than that of similar extracts obtained from the rest of the leech body. A comparable quantitative relationship is found to exist between head and body extracts when tested for another property, *e. g.*, their power to spread in the rabbit skin. 3. The parallelism in the strength of various extracts, as regards both mucolytic activity and spreading power, supports the view that the "mucinase" and the leech spreading factor may be identical. 4. A mucoprotein has been prepared from normal rabbit skin. 5. The viscosity of a skin mucoprotein solution is rapidly and considerably reduced by the action of leech extracts. 6. The effect of leech extracts on the skin mucoprotein *in vitro* suggests that their ability to spread through the skin may be due, at least in part, to their power to cause hydrolysis or depolymerisation of the same or a similar compound *in vivo*.

[†] The name "mucoprotein" refers especially to the physical properties of the material since the solution may contain different soluble components of the skin.

[8] Meyers, K., Hobby, G. L., Chaffee, E., and Dawson, M. H., *J. Exp. Med.*, 1940, **71**, 137.

11304

Effect of Synthetic Vitamin K Compounds on Prothrombin Concentration in Man.

P. M. AGGELER, S. P. LUCIA AND LEON GOLDMAN.

*From the Departments of Medicine and Surgery, University of California Medical School, San Francisco, California.**

The effectiveness of preparations of vitamin K in correcting hypoprothrombinemia has been demonstrated by numerous investigators.[1] Following the discovery of the antihemorrhagic activity of certain naphthoquinones,[2] several synthetic crystalline compounds were made available for clinical use in man. Butt, *et al.*,[3, 4] administered 2-methyl-3-hydroxy-1,4-naphthoquinone (phthiocol) in doses of 25 to 50 mg intravenously to 9 patients with obstructive jaundice or disease of the liver; and 1,4-dihydroxy-2-methyl-naphthaldehyde in doses of 5 to 10 mg intravenously to 10 patients with obstructive jaundice. These preparations were effective in shortening the prolonged prothrombin time as calculated by the method of Quick. No untoward reactions were observed.

---

* Assisted by a grant from the Christine Breon Fund.

[1] Quick, A. J., *Am. J. Med. Sci.*, 1940, **199**, 118.

[2] Almquist, H. J., and Klose, A. A., *J. Am. Chem. Soc.*, 1939, **61**, 1293.

[3] Butt, H. R., Snell, A. M., and Osterberg, A. E., *Proc. Staff Meet. Mayo Clin.*, 1939, **14**, 497.

[4] Snell, A. M., and Butt, H. R., *J. A. M. A.*, 1939, **113**, 2056, footnote 41.

We have studied the effectiveness of 3 synthetic naphthoquinones—2-methyl-3-hydroxy-1,4-naphthoquinone (phthiocol), 2-methyl-1,4-naphthoquinone, and 4-amino-2-methyl-naphthol-hydrochloride—in 26 patients, 11 having obstructive jaundice, 11 chronic diseases of the liver, 2 acute diseases of the liver, 1 non-tropical sprue, and 1 gastro-colic fistula. The prothrombin concentration was determined by the method of Quick.[5] All determinations were made in duplicate.

Phthiocol† was administered intravenously in doses of 50 to 300 mg of a 0.5% solution. 4-amino-2-methyl-naphthol-hydrochloride‡ was administered intravenously in doses of 5 to 30 mg of a 0.1% solution. Gelatin capsules containing 0.6 mg of 2-methyl-1,4-naphthoquinone‡ dissolved in corn oil were administered orally in daily doses of 3.6 to 13.2 mg. The total daily dose was divided into 3 equal parts and given after meals. With the exception of Case 25, 100 mg of desoxycholic acid was given with each 1.2 mg of 2-methyl-1,4-naphthoquinone. Bile salts were not used in Case 25 nor given to those patients who received the intravenous preparations.

Results. A. *Obstructive Jaundice.* (Table I.)

The prothrombin concentration before treatment varied from 3% to 45%, and 24 hours after treatment from 60% to 130%. The duration of the significant effect produced by the intravenous compounds lasted in some cases as long as 7 days. The administration of 50 mg phthiocol (Case 10) elevated the prothrombin concentration to 50% in one hour, 60% in 4 hours, and 100% in 24 hours. After the intravenous administration of 10 mg of 4-amino-2-methyl-naphthol-hydrochloride (Case 6), the prothrombin concentration was elevated to 30% in 1 hour, 50% in 4 hours, 75% in 8 hours, and 100% in 24 hours. In Case 1 the prothrombin concentration was maintained at 100% for 3 months on a daily oral dose of 0.6 mg of 2-methyl-1,4-naphthoquinone. The relatively poor response observed in Case 4 may have been due to a severe diarrhea from which this patient suffered. In Case 6 following exploratory laparotomy, a rapid diminution in the prothrombin concentration was observed.

B. *Chronic Diseases of the Liver.* (Table II.)

---

[5] Quick, A. J., and Grossman, A. M., Proc. Soc. Exp. Biol. and Med., 1939, **41**, 227.

† Made available through the courtesy of the Galen Company, Berkeley, California.

‡ Made available through the courtesy of Parke, Davis and Co., Detroit, Michigan.

TABLE I.
Obstructive Jaundice.

				Prothrombin concentration						
					On day following institution of treatment					
Case No.	Diagnosis	Compound administered	Dosage mg	Before treatment %	1 %	2 %	3 %	4 %	5 %	6 %
1	Metastatic carcinoma of common bile duct	2-methyl-1,4-naphthoquinone	3.6 daily	35	70	—	85	—	90	—
2	Stricture of common bile duct	”	5.4 ”	40	75	95	100	—	—	100
3	Stone in common bile duct	”	3.6 ”	40	80	90	—	85	100	—
4	Carcinoma of head of pancreas	”	5.4 ”	45	60	70	60	—	—	—
5	Metastatic carcinoma of common bile duct	4-amino-2-methyl-naphthol-HCl	10	30	130	—	125	—	130	—
6	Carcinoma of head of pancreas	”	10	25	100	90*	50	55	50	—
7	Stone in common bile duct and multiple pyogenic abscesses of the liver	”	5	3	75	Patient died				
8	Metastatic carcinoma of common bile duct	Phthiocol	50	10	—	95	—	95	—	90
9	Carcinoma of ampulla of Vater	”	50	30	70	—	60	—	50	—
10	Stone in ampulla of gall bladder	”	50	40	100	110	90	90	—	75
11	Metastatic carcinoma of common bile duct	”	50 followed by 50 mg on 2d day	30	65	65	75	—	60	70

*Exploratory laparotomy on this day.

TABLE II.
Chronic Liver Disease.

Case No.	Diagnosis	Compound administered	Dosage mg	Prothrombin concentration Before treatment %	On day following institution of treatment 1 %	2 %	3 %	4 %	5 %	6 %
12	Portal cirrhosis	2-methyl-1,4-naphthoquinone	13.2 daily	50	40	—	40	45	40	35
13	,, ,,	,,	5.4 ,,	45	35	40	—	—	—	35
14	Banti's disease	,,	5.4 ,,	30	35	30	—	35	35	—
15	Portal cirrhosis	4-amino-2-methyl-naphthol-HCl	30	50	50	—	50	—	—	50
16	,, ,,	,,	30	60	60	60	60	—	—	—
17	,, ,,	,,	30	25	50	65	60	50	50	‡
18	Primary carcinoma of the liver	,,	10	30	50	45	‡			
19	Portal cirrhosis	Phthiocol	50	35	35	40	—	50	55	65
20	,, ,,	,,	50	40	45	45	45	50	40	40
20*	,, ,,	,,	300	45	40	35	—	45	—	40
21	Primary carcinoma of the liver	,,	50	40	55	—	55	—	65	—
21†	,, ,, ,, ,, ,,	,,	50 daily	55	50	65	50	‡		
22	Portal cirrhosis	,,	50	55	55	—	55	55	50	—
			followed by 150 mg on third day							

*21 days after first injection.
†11 ,, ,, ,, ,,
‡Patient died.

The prothrombin concentration before treatment varied from 25% to 60% and 24 hours after treatment from 35% to 60%. The maximum prothrombin concentration noted in any patient during the 6-day period following the institution of treatment was 65%. There was no significant rise in the prothrombin concentration following treatment in 8 of the 11 patients studied. There was a slight elevation in the prothrombin concentration in 3 cases. These patients were moribund and died within 24 hours of the last prothrombin determination. Large single doses (Cases 20 and 22), and repeated standard daily doses (Case 21) of phthiocol did not elevate the prothrombin concentration beyond that accomplished with a single standard dose. In Case 12, 3.6 mg of 2-methyl-1,4-naphthoquinone was given orally daily for 1½ months, and no significant variation in the prothrombin concentration was observed.

C. *Acute Diseases of the Liver.* Case 23 (acute cholangeitis, acute hepatitis, and *B. coli* septicemia) received oral daily doses of 3.6 mg of 2-methyl-1,4-naphthoquinone. The prothrombin concentration before treatment was 70% and on the following 6 days it was 65%, 50%, 75%, 80%, 80% and 90%. The relative refractivity and the nature of the response of this patient appeared to be conditioned by the severity of the illness. Case 24 (acute yellow atrophy of the liver) received 50 mg of phthiocol intravenously. The prothrombin concentration before treatment and 8 hours later was 20%. This patient died 12 hours after the last test.

D. *Gastrointestinal Diseases.* Case 25 (non-tropical sprue) received daily oral doses of 3.6 mg of 2-methyl-1,4-naphthoquinone Bile salts were not given. The prothrombin concentration before treatment was 30%, and on the following 6 days it was 55%, 75%, 70%, 70%, 70%, and 75%.

Case 26 (gastro-colic fistula) was treated with 4-amino-2-methyl-naphthol-hydrochloride and phthiocol. The prothrombin concentrations were as shown in Table III.

TABLE III.

Days	Prothrombin conc., %	Days	Prothrombin conc., %	Days	Prothrombin conc., %
1*	50	9	60	21§	70
2	75	10	60	22	80
4	55	11	60	23	70
5†	60	12‡	70	25	85
6	55	13	70	28	70
7	75	14	70	36	65
8	75	19	60	43	55

*Single intravenous dose of 10 mg 4-amino-2-methyl-naphthol-hydrochloride.
† ʼʼ ʼʼ ʼʼ ʼʼ 20 ʼʼ 4-amino-2-methyl-naphthol-hydrochloride.
‡ ʼʼ ʼʼ ʼʼ ʼʼ 30 ʼʼ 4-amino-2-methyl-naphthol-hydrochloride.
§ ʼʼ ʼʼ ʼʼ ʼʼ 50 ʼʼ phthiocol.

Summary. Three synthetic vitamin K compounds were administered to 26 patients with hypoprothrombinemia. Following treatment the prothrombin concentration: (1) was markedly elevated in 11 patients with obstructive jaundice, (2) was not elevated in 8 and only slightly elevated in 3 patients with chronic diseases of the liver; (3) was not elevated in 1 patient, and after an initial delay was elevated in 1 patient with acute diseases of the liver; (4) was moderately elevated in 2 patients, one with non-tropical sprue, and the other with gastro-colic fistula. When considered in terms of the dosages employed, there were no significant qualitative differences in the relative effectiveness of the three compounds. No untoward reactions were observed except that the patients receiving large doses of 4-amino-2-methyl-naphthol-hydrochloride complained of slight burning pain at the site of injection.

11305 P

Effect of Posterior Pituitary Extract on Concentration of Urine Secreted during Osmotic Diuresis in Rabbit.*

WILLIAM G. PAINE AND ERWIN E. NELSON.

From the Department of Pharmacology, Tulane University of Louisiana School of Medicine.

When 10% sucrose is infused rapidly into the circulation of anesthetized rabbits, there follows the rapid elimination of a large amount of urine, having a sugar concentration approaching that of the infusion fluid, and almost keeping pace with it as to rate. The injection of posterior pituitary extract during this diuresis results in a further acceleration of rate. This has been shown to be due to an increase in glomerular filtration.[1] If from the calculated filtration, using the amount of sucrose in plasma and urine for making the calculation, there be subtracted the amount of urine actually eliminated, there is obtained a figure which represents the amount of filtrate reabsorbed during the passage through the tubules. During this phase of increase in filtration from pituitary extract, there not only is no decrease in absorption, but there may actually be a slight increase. During this pituitary "diuresis" the sugar content of urine falls

---

* Supported in part by Grant No. 315 from the Council on Pharmacy and Chemistry of the American Medical Association.

[1] Nelson, E. E., *J. Pharm. Exp. Therap.*, 1934, **52**, 184.

while that of the chlorides rises. With passing off of the pituitary effect, the concentrations return to or toward their original levels. In a consideration of these changes the following view was expressed: "It is possible that with these high rates of filtration the maximum absorbing capacity of the tubules is approached, so that changes in filtration from various experimental procedures can be observed in a relatively uncomplicated form."[1]

In the previous report no estimates were made of the changes in osmotic concentration of the urine during the changes in filtration. In the present study changes in depression of the freezing point have been used to indicate changes in osmotic concentration. The finding that these values are relatively constant has been taken as supporting the suggestion of the earlier report, namely that under the conditions of experiment the maximum concentrating capacity of the tubules is approached.

Experimental. Large rabbits, under urethane anesthesia, were given 10% sucrose solution intravenously by means of the intravenous infusion apparatus recently described from this laboratory.[2] Urine was collected into graduated centrifuge tubes for 5-minute intervals. The rate of secretion always exceeded one cubic centimeter per minute. Chlorides were determined by a modified Volhard-Arnold method, specific gravity by weighing in a one cubic centimeter pyknometer, depression of freezing point using a Beck-

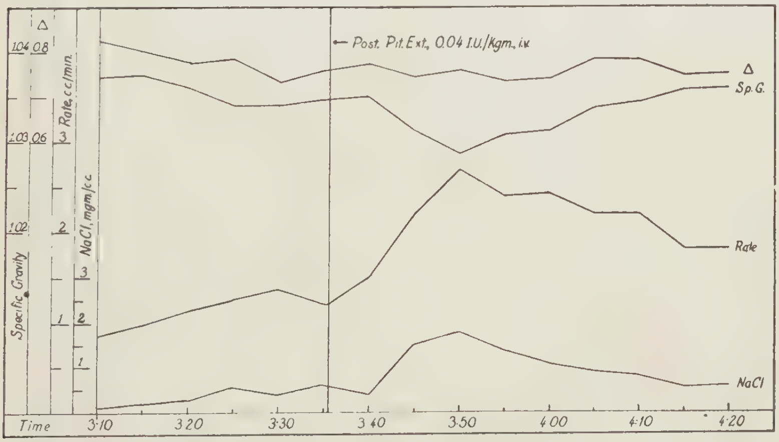


FIG. 1.

Effect of injection of posterior pituitary extract on rate, chloride content, specific gravity, and freezing point depression, of urine formed during continuous intravenous infusion of 10% sucrose solution, in a rabbit. Morphine-urethane anesthesia.

---

[2] Katz, Gerhard, *Science*, 1939, **89**, 63.

man thermometer in the usual manner. When the rate had been constant for at least 4 5-minute periods, posterior pituitary extract was given intravenously, in amounts of the order of 0.04 International Units per kg body weight.

As in the previous study, this amount of pituitary was followed almost invariably by an increase in both rate and chloride content. Sucrose determinations were not made, but in the previous series it invariably fell. Specific gravity either fell or remained constant, and the freezing point remained practically constant. The results, which were consistent throughout, are illustrated in the record of a single experiment shown in Fig. 1.

Conclusions. The finding that the osmotic concentration, as revealed by the depression of the freezing point, remains constant during the action of pituitary extract on the diuresis induced by rapid intravenous infusion of sucrose solutions, lends support to the view that the absorption capacity of the tubules has been reached under these conditions of experiment.

11306

Etiology of Hypertension Due to Complete Renal Ischemia.

MYRON PRINZMETAL,* HARVEY LEWIS AND SIDNEY LEO.† (Introduced by Ella H. Fishberg.)

From the University of Southern California Medical School.

It is generally agreed that the hypertension which develops in experimental animals as a result of partial[1] or complete[2] ischemia of the kidneys is of humoral and not of nervous origin.

Although *renin,* a pressor extract prepared from the renal cortex, has been implicated by some observers,[3, 4] neither this product nor any other has to date been proved to be the substance responsible for ischemic hypertension.

---

* Dazian Fellow.

† Beaumont Research Fellow.

[1] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[2] Taquini, A. C., *Rev. Soc. argent. de biol.*, 1938, **14**, 422.

[3] Prinzmetal, M., and Friedman, B., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 122.

[4] Harrison, T. R., Blalock, A., and Mason, M. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 38.

In this communication we wish to report the finding of a perfusate of ischemic kidneys which causes the elevation of blood pressure following the release of complete renal ischemia. Until the precise nature of the active principle has been determined, we propose the name *ischemin*, which may be defined as the pressor substance contained in perfusates of completely ischemic kidneys and which is responsible for the hypertension resulting from the release of total renal ischemia. For convenience, the renal perfusate containing the pressor substance will also be termed *ischemin*.

The results of the observations made thus far are summarized briefly.

In 8 experiments, cats were anesthetized with ether and the renal pedicle of one kidney clamped. After 4 to 6 hours the animal was reanesthetized with nembutal, the blood pressure recorded from the carotid artery, and both the normal and ischemic kidneys removed. Within 5 minutes following the removal of the kidneys, both renal arteries were cannulated and the kidneys perfused with 1 cc of warm saline per gram of kidney. The perfusates were then injected intravenously into the same animal. The perfusates of the ischemic kidneys uniformly gave a marked and prolonged rise in blood pressure, amounting to as much as 100 mm of Hg, whereas those obtained from the normal kidneys had no pressor effect.

Perfusates of ischemic hind limbs of cats were found to contain no pressor substance.

The first series of experiments having demonstrated the presence of pressor material *(ischemin)* in perfusates of ischemic kidneys, it was decided to determine whether this substance was responsible for the hypertension resulting from the release of complete renal ischemia (Taquini[2]). If *ischemin* causes this rise in blood pressure, there would necessarily be less *ischemin* in the perfusate of the kidney, the circulation of which has been reëstablished than in a control ischemic kidney in which the circulation has not been reëstablished. For this reason the following experiment was devised:

Both renal pedicles of 22 cats were clamped under ether anesthesia. After 4 to 6 hours, the animals were reanesthetized, with nembutal and the blood pressure recorded from the carotid artery. In each instance, upon reëstablishing the circulation of one kidney, the usual rise in blood pressure took place.[2] The other kidney was not unclamped. Fifteen minutes later both kidneys were removed and perfused in the manner previously described. The perfusate of the released kidney was injected intravenously into the same animal, followed after a suitable interval by the perfusate of the unreleased kidney.

It was found that the perfusate of the unreleased kidney showed a significantly greater pressor effect than that of the released kidney, the average rise in blood pressure being 33 mm and 16 mm of Hg, respectively.

The smaller pressor response of the perfusate of the released kidney, as compared with that of the unreleased kidney, is readily explained by the washing out into the general circulation of some (or all) of the *ischemin* from the released kidney upon removal of the clamp.

The perfusate of the released kidney was intentionally injected first, because of the phenomenon of tachyphylaxis (see *ischemin* characteristics a). Except for this phenomenon, the difference in pressor response would have been considerably greater.

Additional evidence that *ischemin* is the substance responsible for the hypertension occurring after reëstablishment of the circulation of completely ischemic kidneys follows:

1. 933F (piperidomethyl-3-benzodioxane) does not reverse the rise in blood pressure following reëstablishment of the circulation of completely ischemic kidneys or the pressor response to *ischemin.*

2. The blood pressure curve following reëstablishment of the circulation of completely ischemic kidneys is similar to that produced by intravenous injection of *ischemin.*

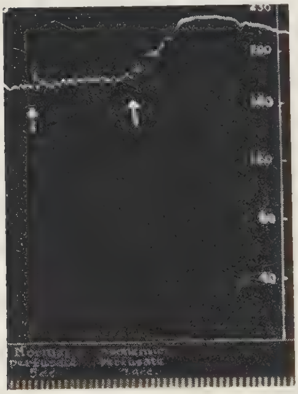


Fig. A.

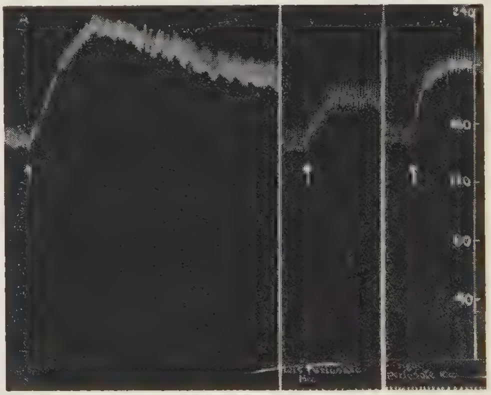


Fig. B.

Fig. A.

At first arrow, intravenous injection of perfusate of normal right cat's kidney. At second arrow, injection of perfusate of same animal's left kidney rendered completely ischemic for 5 hours.

Fig. B.

Five hours previously, both renal pedicles clamped. At first arrow, clamp removed from left renal pedicle. At second arrow, intravenous injection of perfusate of left (released) kidney. At third arrow, injection of perfusate of right (unreleased) kidney.

Nembutal anesthesia. Time 15-second intervals.

TABLE I.

Time	Pressor effect
3:24	40 mm Hg
3:50	32
4:17	28
4:27	24
4:33	14
4:43	14
5:05	12

The amount of *renin* in extracts of normal, unreleased ischemic, and released ischemic kidneys is now being investigated.

Ischemin exhibits the following characteristics:

a. Tachyphylaxis. By this is meant that repeated injections of a pressor substance into the same animal cause decreasing effects. An example in the case of *ischemin* is shown in Table I.

b. The boiling of *ischemin* for 5 minutes destroys the active pressor principle.

c. The action of *ischemin* is not reversed by 933F (piperidomethyl-3-benzodioxane).

From these experiments it is concluded that as a result of complete renal ischemia, pressor material *(ischemin)* accumulates in the kidney and is readily washed out into the general circulation when the renal blood flow is reëstablished or by artificial perfusion of the extirpated organ. This is the first time a pressor substance has been obtained which has been proved to be the cause of hypertension due to renal ischemia.

This substance *(ischemin)* has certain properties in common with *renin:* destruction by heat, similar character of the pressor curves, the property of tachyphylaxis, and failure of 933F to abolish the pressor reaction,[5] but until decisive physiological and chemical studies have been performed, no definite statement should be made concerning the relationship of these two products, nor to the heat-stable renal pressor substance which has recently been described.[6]

---

[5] Katz, L. N., and Friedberg, L., *Am. J. Physiol.*, 1939, **127**, 27.

[6] Victor, J., Steiner, A., and Weeks, D. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 767.

11307 P

Method for Obtaining Group A Hemolytic Streptococci Sensitive to Reaction of Capsular Swelling.

A. RINFRET. (Introduced by Arthur F. Coca.)

From the Research Department of Lederle Laboratories, Inc., Pearl River, New York.

The following experiments are an approach to the problem of mouse virulence and type-specificity of Lancefield Group A beta hemolytic streptococci from the standpoint of encapsulation and the reaction of capsular swelling with homologous serum. Many of our stock cultures were avirulent with little or no demonstrable capsules. Attempts to produce capsular swelling with these organisms were unavailing. Further, although capsules were at times noted on our most virulent cultures, all attempts to produce capsular swelling were negative.

It has been shown by Dawson,[1] Seastone,[2] Loewenthal[3] and others that encapsulation of hemolytic streptococci is an attribute always accompanying highly virulent invasive strains. Loewenthal[3] regarding the encapsulated organism as capable of evoking protective antibodies used only this type of organism in producing protective antisera. He succeeded in making antisera from mucoid and non-mucoid strains, but in either case he emphasized the presence of capsules. In an earlier paper[4] he noted the "swelling" of these capsules with the addition of homologous serum to the organisms.*

The logical approach to the problem of increasing virulence is by mouse passage. For our purposes this method offered certain difficulties. Parish and Okell,[5] Pulvertaft,[6] and Hartley[7] have shown that streptococci elaborate toxins which are lethal for mice. Todd[8] further showed a limited protection with an antitoxin (antistreptolysin O) against living cultures of streptococci injected into mice. From these experiments it is clear that toxemia may, in large measure, be responsible for the deaths of mice injected with living streptococci.

---

[1] Dawson, M. H., Hobby, G. L., and Olmstead, M., *J. Inf. Dis.*, 1938, **62**, 138.

[2] Seastone, C. V., *J. Exp. Med.*, 1939, **70**, 347.

[3] Loewenthal, H., *Brit. J. Exp. Path.*, 1938, **19**, 143.

[4] Loewenthal, H., *Brit. J. Exp. Path.*, 1934, **15**, 298.

* It should be noted that these organisms fall serologically into Lancefield's Group B.

[5] Parish, H. J., and Okell, C. C., *J. Path. Bact.*, 1927, **30**, 521.

[6] Pulvertaft, R. J. V., *J. Path. Bact.*, 1928, **9**, 276.

[7] Hartley, P., *Brit. J. Exp. Path.*, 1928, **9**, 259.

[8] Todd, E. W., *Brit. J. Exp. Path.*, 1938, **19**, 367.

Believing that a death for which a septicemia was entirely responsible would yield an organism better suited to our purpose, scarlet fever antitoxin was injected simultaneously with culture into the mouse peritoneum. It was thought that the inhibition of toxin production or the neutralization of toxin as quickly as it was formed would accomplish the desired result.

Procedures. 1. *Mouse Passage.* Thirteen strains originally obtained from Griffith and representing 13 serological types in his classification (types 1, 2, 3, 9, 10, 12, 13, 14, 15, 23, 25, 26, 28) were used. Cultures were taken from vacuum tubes planted in beef-infusion broth with 10% of normal horse serum and incubated at 37° for 18 hours. Five-tenths of a cubic centimeter of the culture, in each case, was inoculated into another tube of serum broth and incubated at 37°C for 4 hours; 0.5 cc of the dilution containing approximately one lethal dose was injected simultaneously with 0.5 cc of unrefined scarlet fever antitoxin† into a mouse. The intraäbdominal route was used.

Immediately upon the death of the mouse (5 to 24 hours) the spleen was excised aseptically and macerated in 1 cc of sterile peptone water. To one-half cc of this suspension, one-half cc of antitoxin was added. This mixture was immediately reinjected into mice.

After excision of the spleen the heart's blood was inoculated into serum broth. When growth was sufficient, a capsular swelling preparation was made and the reaction noted.

Upon the death of the mice injected with the splenic material, the culture was again tested for capsular swelling. When positive reactions were consistently obtained, the cultures were put under vacuum and stored at 4°C.

2. *Action on horse blood agar.* Stock cultures and antitoxin-treated cultures were plated and any variations noted. The stock culture plate served as the control.

3. *Growth in serum broth.* Cultures were grown in serum broth for varying periods of time at 37°C and room temperature (1 to 72 hours), the macroscopic and microscopic conditions of the growth were noted. Microscopic examination was made primarily to ascertain the presence or absence of capsules.

4. *Capsular swelling preparations.*

a. 1 mm loop of culture.

---

† 200 to 500 units U.S.P.H.S. or 10,000 to 25,000 original neutralizing units.

b. 5 mm loop of antiserum.
c. 5 mm loop of methylene blue.
d. Incubation at 52° for varying periods of time (½ to 24 hrs).
e. Control with saline or with heterologous serum for the detection of cross reactions.

Results. The results of these experiments may best be shown by a comparison with beta hemolytic streptococci not so treated. The treated organisms generally show encapsulation which is readily accentuated by the presence of specific antiserum. The virulence of the treated organism is increased and the hemolytic zone around the colonies is altered. These results are shown in Table I.

Summary. A method of obtaining virulent, encapsulated group A beta hemolytic streptococci, sensitive to the reaction of capsular swelling with homologous antisera, has been described. Essentially this consists of the injection of living streptococci into mice simultaneously treated with unrefined scarlet fever antitoxin.

TABLE I.

Untreated	Treated with Antitoxin
1. Action on horse blood agar.	
a. Hemolytic zone sharply defined and transparent.	*a.* Hemolytic zone frequently not sharply defined and is generally translucent (18 to 24 hours).
b. No pigment was ever noted on base of colony.	*b.* Base of colony frequently showing rose to deep rose shades.
c. Smooth or rough colonies.	*c.* Smooth colonies.
2. Growth in broth.	
a. Often flocculent with sediment.	*a.* Invariably of even turbidity.
b. Capsule not visible in ordinary wet methylene blue preparation.	*b.* Capsule frequently visible in wet methylene blue preparation.
c. Soon loses capsules in broth as shown by dry capsule stains.	*c.* Capsules observed with capsule stains after 48 hours in many cases.
3. Capsular swelling.	
a. Negative with specific antiserum.	*a.* Positive with antiserum. This reaction is unmistakable.
4. Agglutination.	
a. Organisms readily agglutinated with homologous serum using rapid slide agglutination test.	*a.* To date these organisms have proved inagglutinable with homologous serum, using rapid slide test.
5. Virulence for mice.	
a. May or may not be virulent.	*a.* Virulent (10^{-4} to 10^{-9}).
b. If virulent, 10 lethal doses generally kill in 16 to 48 hr.	*b.* 10 lethal doses kill in 5 to 12 hr.
c. Many mouse passages are necessary to build virulent strains.	*c.* With strains so far employed a relatively few passages (1 to 10) are required to obtain the desired virulence.

11308 P

Origin of L Type Colonies in Bacterial Cultures.*†

L. Dienes.

From the Departments of Pathology and Bacteriology, Massachusetts General Hospital, Boston, Mass.

Small secondary colonies, corresponding morphologically to young colonies of the L1 strain isolated by Klieneberger from cultures of *Streptobacillus moniliformis,* have been observed occasionally in cultures of various Gram negative bacteria.[1] In all cultures in which such secondary colonies developed a large number of bacteria swelled up into large deeply stained, spherical or fusiform bodies. It was first seen in a culture of *Bacillus influenzæ* that occasionally L type of colonies developed from the large bodies. Later this process was observed repeatedly in the cultures of 2 colon bacillus strains.

After 24 hours of growth the colonies of these strains consisted mainly of large spherical bodies. In order to observe the development of these large bodies in these 2 strains different procedures proved to be helpful. The colonies of one strain were very tenacious and when an impression of the colonies was made on blood agar plates only the large bodies, with very few bacteria, were transferred. After 6 to 12 hours fine curved filaments grew from the large bodies into the agar and after 24 hours about two-thirds of them developed into tiny L type colonies. With the other strain a similar process was observed when a broth culture was transferred on blood agar plates after varying time intervals. In the transplant made after 24 hours' growth many large bodies were visible on the agar surface. Only a few bacterial colonies developed in these plates but after 6 to 12 hours the large bodies began to germinate and produced L type colonies. Transplants made from the broth culture after 48 hours, gave only abundant bacterial growth. With either strain, L type colonies developed only at the places where large bodies were present. Therefore, it is extremely unlikely that the L type colonies develop from invisible granules attached accidentally to the large bodies or from regularly shaped bacteria. In transplants made with platinum loops from agar cultures the development

---

* The expenses of this investigation have been defrayed, in part, by a grant from the Commonwealth Fund.

† This is publication Number 43 of the Robert W. Lovett Memorial Fund for the study of crippling disease, Harvard Medical School, Boston, Mass.

[1] Dienes, L., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 636.

of the large bodies into the L type colonies was difficult to demonstrate because the agar was overgrown by bacteria.

Subsequent to these observations it was found that the germination of the large bodies of *Streptobacillus moniliformis* could be observed if appropriate media were employed. The best results were obtained with an alkaline meat infusion agar (pH 8.2) without the addition of blood or ascitic fluid. A freshly isolated strain of *Streptobacillus moniliformis* was used. After 24 to 36 hours' growth on ascitic fluid media the colonies of this strain consisted mainly of large spherical bodies. On the alkaline agar macroscopic growth was observed only in thickly seeded areas. This growth consisted of regular *Streptobacilli* showing little pleomorphism. The remains of the abundant transplant degenerated and could not be stained. In the thinly seeded areas bacterial growth was absent but the large bodies remained well preserved and deeply stained. From a large number of them tiny L1 colonies were seen to grow in a manner similar to that noticed in the colon bacillus cultures. No L type colonies developed in the absence of the large bodies. Temperature between 25-30°C was most favorable for the germination of the large bodies.

According to these observations there are two stages in the development of L colonies. One first notes that the bacteria swell and form large deeply stained bodies. Under appropriate cultural conditions these germinate and produce the L type colonies. In cultures of L1 strains growth may be transmitted by small filter-passing elements. The transition from regular bacilli into the L form seems to occur through the large bodies. The significance of these tiny colonies, consisting often of very small elements, developing from the bacteria in this peculiar way, is very puzzling. The L1 strains are antigenically similar to the *Streptobacillus moniliformis*[3, 4] and occasionally revert to the typical *Streptobacillus*.[2, 4] These studies leave no doubt that the L1 strain is a variant form of the *Streptobacillus* and not an extraneous element in the cultures. In the case of other bacteria we can infer only by analogy that they represent a variant form.

---

[2] Dienes, L., *J. Inf. Dis.*, 1939, **65**, 24.

[3] Klieneberger, E., *J. Hyg.*, 1938, **38**, 458.

[4] Dawson, M. H., and Hobby, G. L., *Transactions Assn. Am. Phys.*, 1939, **44**, 329; Third International Congress for Microbiology, Abstracts of Communications, New York, 1939.

11309 P

Reversibility of Quellung Phenomenon on Addition of Type-Specific Polysaccharide.*

W. J. Nungester and Alice H. Kempf.

From the Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.

Although pneumococci can be typed directly from sputum, it has been recognized that the results are sometimes unsatisfactory. Taplin, Meneely and Hettig[1] have outlined an improved method using fresh sputum to obtain the Quellung phenomenon. Essentially, this method consists of breaking up and suspending the sample in saline, centrifuging, and using the sediment for typing. The authors suggested that the water-soluble capsular polysaccharide might well be the interfering substance, which was removed by their technic.

In work published elsewhere,[2] it has been determined that in undiluted samples of ground lung from pneumonic rats, pneumococci present did not give the Quellung reaction with specific antiserum. The reaction did occur if the material were sufficiently diluted with PSS. Heidelberger[3] has shown that excess of antigen prevents visible evidence of antigen-antibody reaction, and has ably discussed the reversibility of the precipitin and agglutinin reactions. Since the above findings indicated that excess antigen prevented the occurrence of the Quellung reaction, it seemed that reversibility of this phenomenon might likewise take place. Hence, the following experiments were performed.

In each of 4 serological tubes were placed 0.1 cc of a broth culture of Type I pneumococcus and 0.1 cc of undiluted type-specific rabbit antiserum; after a short interval, the pneumococci were examined for the presence of swollen capsules. These were uniformly present. Then 0.1 cc of dilutions of Type I polysaccharides were added to each tube to give final amounts of 1.0 mg, 0.1 mg, 0.01 mg, and 0.001 mg in 0.3 cc of fluid, respectively. Five minutes later, the preparations were reëxamined. In the first 2 tubes, the swollen capsules had disappeared. In the last 2 tubes, no change was observed.

A similar reaction was seen employing Type III pneumococcus. However, the Type III specific polysaccharide was partially effective

---

* These studies were aided by a grant from the Horace H. Rackham School of Graduate Studies.

[1] Taplin, G. V., Meneely, G. R., and Hettig, R. A., *J. A. M. A.*, 1938, **111**, 410.

[2] Kempf, A. H., and Nungester, W. J., *J. Infect. Dis.*, 1939, **65**, 1.

[3] Heidelberger, M., *Bact. Rev.*, 1939, **3**, 49.

in causing this reversibility, when 0.01 or even 0.001 mg of the substance was added to the tubes containing previously swollen capsules. The specificity of this reversibility was indicated by the fact that addition of Type I polysaccharide failed to reverse the reaction.

11310

Effect of Sulfapyridine, Sulfathiazole and Sulfamethylthiazole upon Severe Staphylococcal Infection in Mice.*

ELEANOR A. BLISS† AND EARL OTT. (Introduced by P. H. Long.)

From the Biological Division of the Department of Medicine, The Johns Hopkins University Medical School and The Johns Hopkins Hospital, Baltimore, Maryland.

In previous reports[1, 2] it was shown that while sulfanilamide therapy had a slight effect upon the course of staphylococcal infections in mice, the use of sulfapyridine markedly prolonged the lives of the infected animals. In a few instances the mice treated with sulfapyridine survived 2 and 3 months.[3] Eventually, however, they succumbed, showing at postmortem, abscess formation in the kidneys and liver.

Preliminary studies[4, 5] in which the *in vitro* effect of sulfathiazole was tested, indicated that this compound inhibited the growth of a number of microörganisms, including staphylococci, to a greater degree than did sulfanilamide and sulfapyridine. It was then considered worthwhile to compare the activity of this compound and of sulfapyridine in the treatment of severe staphylococcal infections in mice. Later the activities of sulfathiazole and sulfamethylthiazole were similarly compared.

Methods. The mice were infected by the intravenous injection of heavy broth suspensions of a strain of *Staphylococcus aureus* which had been isolated a year previously from a patient, ill with a lung abscess. The method is given in detail elsewhere.[1]

---

* This study was supported by the Chemical Foundation, Inc., of New York City.

† Fellow in Medicine, The Johns Hopkins University Medical School, on a grant from the Calco Division of the American Cyanamid Company.

[1] Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., *Bull. Johns Hopkins Hosp.*, 1938, **62**, 565.

[2] Bliss, E. A., and Long, P. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 32.

[3] Unpublished observations.

[4] Lawrence, C. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 92.

[5] Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 324.

TABLE I.

The Effect of Diets Containing 1% Sulfapyridine, 1% Sulfathiazole or 1% Sulfamethylthiazole upon the Survival Time of Mice Infected with *Staphylococcus aureus.*

	*Series A			†Series B			Series A		Series B	
	No. of mice						No. of mice			
	30	49	50	19	30	30	20	20	30	30
Day of experiment	Controls	Sulfapyridine	Sulfathiazole	Controls	Sulfathiazole	Sulfamethylthiazole	Sulfapyridine	Sulfathiazole	Sulfathiazole	Sulfamethylthiazole
	No. of mice dying						Avg daily consumption of drug—mg/mouse			
—2	(before infection)						not measured		20.2	18.9
—1							22.5	22.4	26.6	24.7
1	14	11	4	3	0	0	6.5	14.6	14.2	14.3
2	1	3	4	0	2	1	21.7	31.6	23.5	22.0
3	4	1	1	7	1	1	23.2	26.1	29.5	23.9
4	3	1	0	4	0	3	29.9	30.5	28.7	29.3
5	2	3	1	2	1	1	30.1	30.6	31.2	30.5
6	0	1	1	1	0	0	27.6	30.8	28.2	31.2
7	3	4	2	2	1	0	not measured		29.0	27.8
8	2	9	1		1	0			32.5	28.3
9	0	5	10		0	1			29.3	30.6
10	0	3	7		0	2			26.1	29.3
11	0	6	3		1	0			25.2	23.2
12	0	1	1		0	0			26.6	27.7
13	0	1	10		1	2			25.4	24.5
14	1		2		1	1			27.3	26.4
15			1		1	2				
16			0		4	1	Avg per mouse/day			
17			0		1	1	23.0	26.6	26.8	25.6
18			0		1	5				
19			0		1	3				
20			1		6	1				
21			0		3	1				
22			0		1	1				
23			0		0	2				
24 to 30			0		0	0				
Survivors (30 days)	0	0	1	0	3	1				
Mean survival time (days)	2.86	5.86	9.04	3.18	16.03	14.20				
Median survival time (days)	2.0	7.0	9.1	2.9	17.0	16.0				
σ	3.07	3.78	5.19	1.7	7.46	6.90				
Standard error of mean	0.56	0.54	0.74	0.38	1.36	1.26				
Standard error of median	0.70	0.68	0.93	0.49	1.70	1.58				

---

*Series A is a summary of 3 experiments started on 12/13 and 14/39 and 1/5/40. The mice received 2 billion, 175 million and 2 billion cocci on the respective dates. Therapy maintained 7 days after infection. Drug consumption was determined only in last experiment.

†Series B is a summary of 2 experiments started on 1/25 and 26/40. The mice received 2 billion and 6 billion cocci on the respective dates. Therapy maintained for 2 wks after infection. Drug consumption was determined in both experiments.

Treatment was carried out by administering the drug in the animals' diet. This method was selected because it is the simplest and at the same time, as McKee and her associates pointed out,[6] the surest means of maintaining a fairly even concentration of these drugs in the blood of the mice. The diet consisted of well ground Purina Dog Chow. The drug was added to this in an amount sufficient to give a 1% concentration and the two were mixed carefully and thoroughly. The mice were kept in individual cages. The drug-diet mixture, offered in containers as described by Bieter, *et al.*,[7] was weighed daily in order to ascertain the amount which had been consumed. Treatment was carried out in this way for 2 days prior to infection and, in the first series of experiments, for one week thereafter, in the second for 2 weeks.

Results. The results are shown in Table I. The mice consumed approximately equal quantities of the 3 drug-diets. In the first series of experiments the mean survival times were 2.86 days for the untreated animals, 5.86 days for those treated with sulfapyridine and 9.04 days for those treated with sulfathiazole. These differences are statistically significant. When the median survival times are calculated the difference between the survival times of the sulfathiazole and sulfapyridine-treated animals (9 and 7 days respectively) is less obviously significant. Sulfathiazole and sulfamethylthiazole, as shown in the second series of experiments, were almost equally effective in prolonging the lives of the infected animals. The difference in the results with sulfathiazole in the two experiments is, of course, due to the fact that therapy was maintained twice as long in the second series. It is interesting that in both series the average mouse treated with sulfathiazole survived just 2 days after treatment was discontinued.

Discussion. These results are in accord, as far as the comparison of sulfathiazole and sulfapyridine is concerned, with those of Barlow and Homburger,[8] when allowance is made for the differences in the two technics. They do not agree with these authors' results with sulfathiazole and sulfamethylthiazole since they found sulfamethylthiazole to be the more effective of the two compounds, and we found no therapeutic difference between them. The superiority of sulfamethylthiazole, in the hands of Barlow and Homburger, is probably attributable to the fact that after the first 32 hours in their experiments the drugs were given in single daily doses and as sulfathiazole is excreted more rapidly than its methyl derivative, the blood levels obtained with it would have a lower daily average.

---

[6] McKee, C. M., Rake, Geoffrey, Greep, R. O., and van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 417.

[7] Bieter, R. N., Larson, W. P., Cranston, E. M., and Levine, Milton, *J. Pharm. and Exp. Therap. Proc.*, 1939, **66**, 3.

[8] Barlow, O. W., and Homburger, E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 792.

Summary and Conclusions. Sulfathiazole and sulfamethylthiazole, administered as 1% of the diet showed a distinct and equal therapeutic value in prolonging the lives of mice heavily infected with *Staphylococcus aureus.* Sulfathiazole proved to be somewhat more efficient in this respect than sulfapyridine.

We are indebted to E. R. Squibb and Sons and the Calco Chemical Division of the American Cyanamid Company for the sulfathiazole used in these experiments, and to the Department of Medical Research of the Winthrop Chemical Company for the sulfamethylthiazole.

11311 P

Effect of Pregneninolone (17-Ethinyl Testosterone) on Genital Tract of Immature Female Rats.

U. J. Salmon and A. A. Salmon.

From the Laboratories of the Mount Sinai Hospital, New York City.

It has previously been shown that testosterone, when administered to immature female rats, exhibits 3 biological properties: (a) it is gynecogenic, causing premature opening of the vagina[1] and growth of the epithelial and muscular elements in both the vagina and uterus;[2, 3] (b) it is androgenic, causing growth of the clitoris and preputial glands;[2, 3] (c) it is hypophyseotropic, stimulating the hypophysis to secrete gonadotropic hormone, which is manifested by growth of follicles and appearance of corpora lutea in the ovaries.[3-7]

---

[1] Butenandt, A., and Kudzus, H., *Hoppe-Seyler's Z.*, 1935, **75**, 237.

[2] Korenchevsky, V., Dennison, M., and Hall, K., *Biochem. J.*, 1937, **31**, 780.

[3] Salmon, U. J., *Endocrinology*, 1938, **23**, 779.

[4] Salmon, U. J., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 352.

[5] Nathanson, I. T., Franseen, C. C., and Sweeney, A. R., Jr., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 385.

[6] Starkey, W. F., and Leathem, J. H., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 218.

[7] Freed, S. C., Greenhill, J. P., and Soskin, S., Proc. Soc. Exp. Biol. and Med., 1938, **39**, 440.

Recently, Inhoffen and coworkers[8] have added an ethinyl group to testosterone at the 17th carbon atom, producing a compound (Δ^4 pregnen-in-20-on-3-ol-17; 17-ethinyl testosterone; pregneninolone) which, chemically, is closely related to both testosterone and progesterone. Inhoffen and Holweg[9] have shown that this compound has a progesterone-like action in immature rabbits and is, furthermore, active when administered orally. Ruzicka, Hofmann and Meldahl[10] made essentially similar observations. In humans, the compound has been shown to produce a progesterone-like effect on the estrogen-primed endometrium[11, 12] and to cause uterine bleeding in cyclical and amenorrhoeic women.[13]

The present study was undertaken to determine the biological properties of this compound in the immature, intact and ovariectomized, female rat. The pregneninolone* was dissolved in sesame oil, in concentration of 1 mg per cc of oil, and administered subcutaneously. Twelve littermate, immature, female rats (3 litters), 30 days of age, were injected daily with 0.5 mg of pregneninolone, for 3 days, and sacrificed at the end of 96 hours.

Opening of the vagina occurred on the 3rd day, in all injected animals, after the animals had received a total of 1.5 mg of pregneninolone. Vaginal smears revealed a complete cornification reaction in all. In the animals sacrificed at the end of 96 hours, the ovaries did not reveal macroscopic evidence of follicle stimulation or corpora lutea; the uteri, clitoris and preputial glands, in each case, were definitely enlarged and abundant secretion could be expressed from the latter.

Microscopic examination of serial sections of the ovaries failed to reveal any evidence of follicle stimulation or corpora lutea. On section, the muscular coats of the uteri were found to be moderately hypertrophied, as compared with the controls. Microscopic examination of the vaginae revealed complete cornification of the mucosa and hypertrophy of the muscular coats.

---

[8] Inhoffen, H. H., Longemann, W., and Serini, A., *Ber. Deutsch. chem. Ges.*, 1938, **71**, 1024.

[9] Inhoffen, H. H., and Hohlweg, W., *Naturwissenschaften*, 1938, **26**, 96.

[10] Ruzicka, L., Hofmann, K., and Meldahl, H. F., *Helv. chem. Acta.*, 1938, **21**, 372.

[11] Clauberg, C., and Üstün, Z., *Zentralbl. f. Gynakologie*, 1938, **62**, 1745.

[12] Salmon, U. J., Walter, R. I., and Geist, S. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 252.

[13] Zondek, B., and Rozin, S., *Lancet*, 1939, **1**, 504.

* For the pregneninolone used in this study, we are indebted to Dr. E. Schwenk, of the Schering Corporation, Bloomfield, N. J.

Summary and Conclusions. It appears from this study that Δ^4-pregnen-in-20-on-3-ol-17 exhibits bisexual properties when administered subcutaneously in oil to immature, female rats, having an estrogen-like action on the uterus and vagina and an androgen-like action on the preputial glands and clitoris. The trophic effect on the genital tract appears to be a direct one and not mediated through the ovaries, as shown by the elicitation of similar effects in ovariectomized immature animals. Unlike testosterone, however, pregneninolone appears not to have a stimulating effect upon the gonadotropic activity of the hypophysis, as indicated by the absence of corpora lutea formation or evidence of follicle stimulation in the ovaries.

It is interesting to note that this compound possesses a unique variety of biological properties. In addition to its progesterone-like action on the endometrium, Courrier and Jost[14] have found that pregneninolone will maintain pregnancy in spayed rabbits and is androgenic in castrated rats and chicks. Furthermore, Emmens and Parkes[15] have shown that this compound has estrogen-like properties when administered to adult, female rats, as well as androgenic activity, as indicated by the capon comb growth. It is worthy of note that the introduction of the ethinyl group at the 17th carbon atom of testosterone produced a compound possessing properties that are characteristic of estrogens, androgens and progesterone and at the same time has resulted in a loss of hypophyseotropic potency—the power to stimulate gonadotropic hormone secretion by the hypophysis.

---

[14] Courrier, R., and Jost, A., *C. R. Soc. Biol. Paris,* 1939, **130**, 1162.

[15] Emmens, C. W., and Parkes, A. S., *Nature,* 1939, **143**, 1064.

11312

Local Tissue Reaction to the Implantation of Crystals and Pellets of Estrogenic Hormone.

S. H. GEIST, R. I. WALTER AND U. J. SALMON.

From the Laboratories of the Mount Sinai Hospital, New York City.

Deanesley and Parkes,[1, 2] in a study of the various factors which influence the rate of absorption of sex hormones in animals, demonstrated that the subcutaneous implantation of the pure dry hormone was the most efficient mode of administration. In studies in the human, we have shown[3] that, by means of the subcutaneous implantation of crystals of a-estradiol benzoate, it was possible to prolong the period of effectiveness of the hormone. This was demonstrated by (a) the long period of clinical improvement, (b) the persistence of estrogenic effects on the vaginal smear and (c) the prolonged pituitary inhibition, in menopausal patients.[4] Subsequently, in an attempt to obtain more prolonged effects with the estrogenic substances, pellets of a-estradiol and a-estradiol benzoate were implanted subcutaneously.

One of the questions which immediately arose following the use of this procedure was, what effect the crystalline estrogens would have upon the contiguous subcutaneous tissues. It is our purpose, in this communication, to describe the local tissue reaction to the implanted estrogen crystals* and pellets.*

We have employed the implantation method in a series of 110 cases and from this series 14 patients (of whom 8 were spontaneous menopause, 4 surgical and 2 X-ray castrates), were selected for excision of the implanted hormone and surrounding tissues. Ten patients had been implanted with pellets (5 a-estradiol and 5 a-estradiol benzoate). All of these cases, before the implantation, had typical menopause symptoms and morphologic evidence of estrogen deficiency, as indicated by negative vaginal smears and biopsies of the vaginal mucosa and endometrium. The amount of hormone implanted varied from 15 to 50 mg. The individual pellets weighed

---

[1] Deanesley, R., and Parkes, A. S., *Proc. Roy. Soc. B.*, 1937, **124**, 279.

[2] Deanesley, R., and Parkes, A. S., *Lancet*, 1938, **2**, 606.

[3] Salmon, U. J., Walter, R. I., and Geist, S. H., *Science*, 1939, **90**, 162.

[4] Salmon, U. J., Geist, S. H., and Walter, R. I., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 424.

* For the crystals and pellets of a-estradiol and a-estradiol benzoate used in this investigation, we are indebted to Dr. E. Schwenk of the Schering Corporation, Bloomfield, N. J.

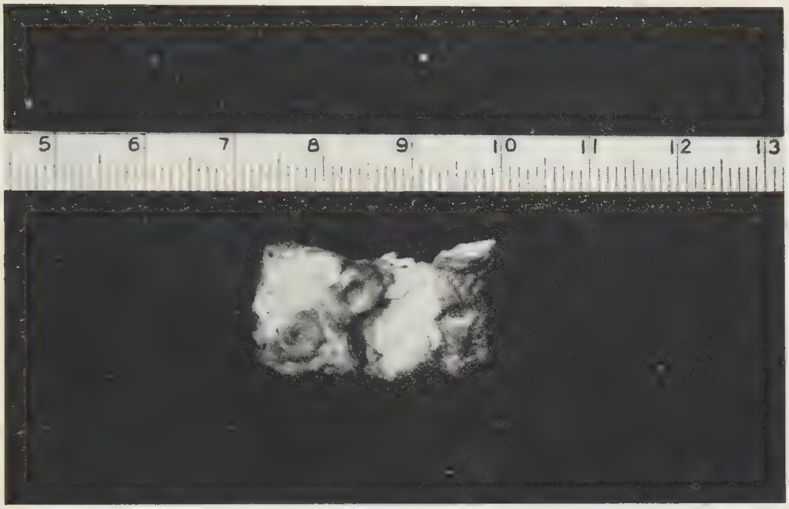


FIG. 1.
Case D.N. Natural size photograph of implantation site, excised 88 days after implantation of 2 pellets of a-estradiol benzoate, showing capsule formation.

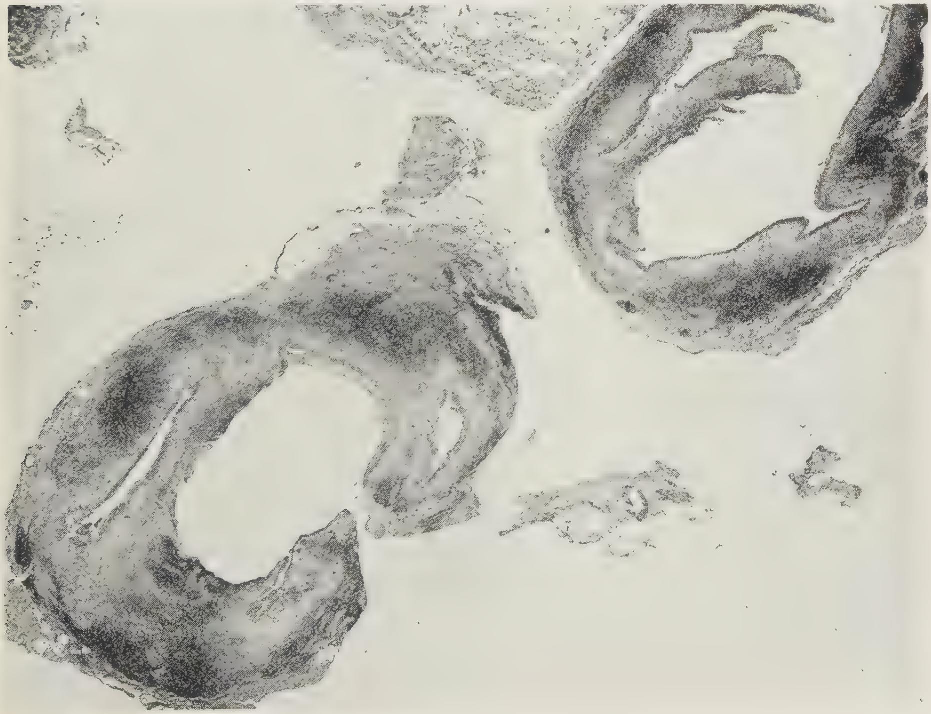


FIG. 2.
Case D.N. Cross section through capsules (× 10).

15 to 25 mg. Four patients were implanted with crystals of a-estradiol and a-estradiol benzoate, varying in weight from 10 to 23 mg. The implantation sites were excised under local (1% novocaine) anesthesia, at periods varying from 23 to 238 days after the implantation. An ample elliptical incision was made over the site of implantation in order to include the skin and surrounding tissues. The pellets were removed and the excised tissue was fixed in 10% formalin. Serial sections were made and stained with hematoxylin and eosin. In none of the cases implanted with crystals were we able to find any gross evidence of the crystals at the time of excision.

It was noted that a fairly uniform tissue reaction occurred in each case. The pellets were completely surrounded by a fibrous capsule which varied in thickness depending upon the duration of implantation. The pellets retained their original shape and consistency and, although intimately adherent to the capsule, could be easily shelled out. The subcutaneous tissues showed a typical foreign body reac-

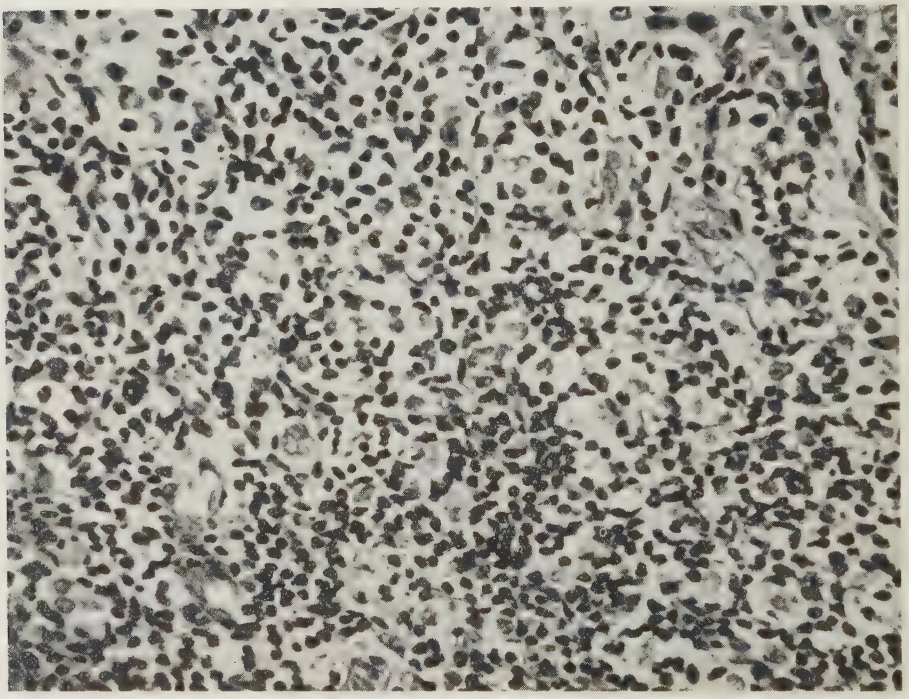


FIG. 3.
Case D.N. Section through middle layer of capsule showing round cells and giant cells (× 400).

tion. Each of the capsules showed essentially the same histologic picture. The protocol of a typical case is presented here.

This patient (D.N.) was implanted in the subcutaneous tissue of the right thigh with 2 pellets of a-estradiol benzoate (total weight, 50 mg) and the implant-site was removed 88 days later. Fig. 1 is a natural size photograph demonstrating the gross appearance of 2 capsules after removal of both pellets. Fig. 2 is a low power magnification of a cross section of the capsules illustrated in Fig. 1, showing the sharply demarcated character and thickness of the capsules. The significance of the thickness of the capsules will be discussed later.

The capsule consists of 3 more or less distinct layers surrounding a central cavity. The inner layer adjacent to the cavity consists of 2 zones: (a) an inner zone which is composed of 1 to 3 layers of cells which are elongated, narrow and eosinophilic and contain irregular nuclei which have no uniform cell position; and (b) an hyalinized connective tissue zone containing scattered lymphocytes, leucocytes

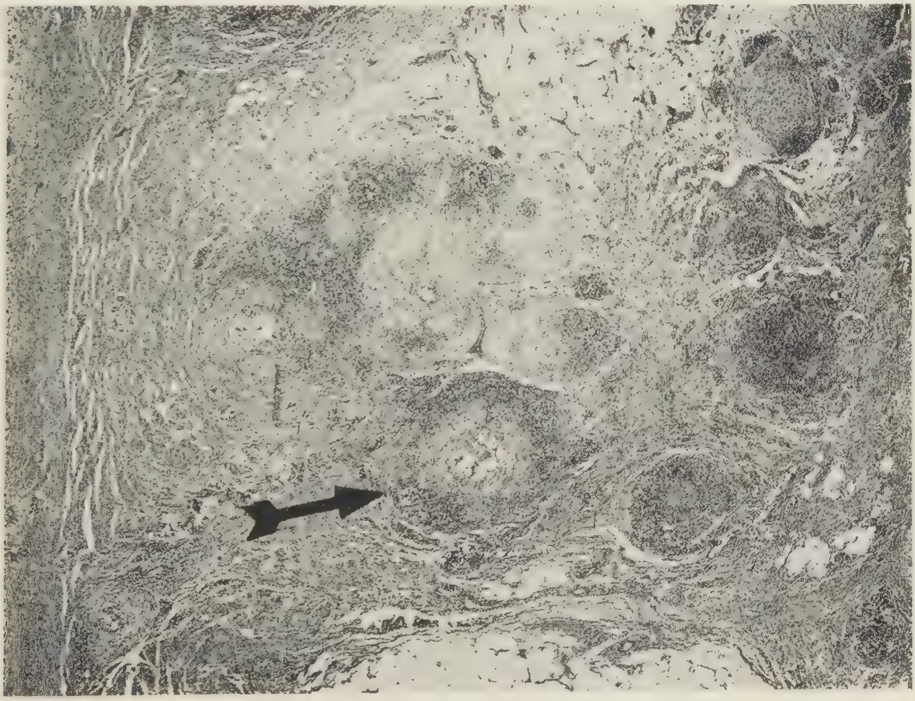


FIG. 4.

Case L.S. Excised tissue 236 days after the implantation of 10 mg of a-estradiol crystals (× 20). Note tubercle-like nodules.

and some large, pale-staining cells with oval nuclei. The nuclei of these large cells have a well-defined chromatin content, the cells resembling phagocytes. The middle layer (Fig. 3) is a slightly wider area, which is supported by an hyalinized connective tissue substratum and is infiltrated by many closely packed, small, round cells (lymphocytes and plasma cells), fibroblasts, occasional leucocytes, and a scattering of giant cells. The outer layer consists of a relatively narrow band of hyalinized fibrous tissue which contains some dilated lymphatic vessels. The surrounding fat tissue shows slight edema and contains a few lymphocytes and giant cells. The skin overlying the implanted pellets is normal.

On excision of the crystalline implantation sites, no macroscopic capsule could be seen. On cut section one could, however, discern multiple, pin-point, grayish-white areas distributed throughout the subcutaneous fat tissue.

Fig. 4 is a low power photograph of a cross section of an im-

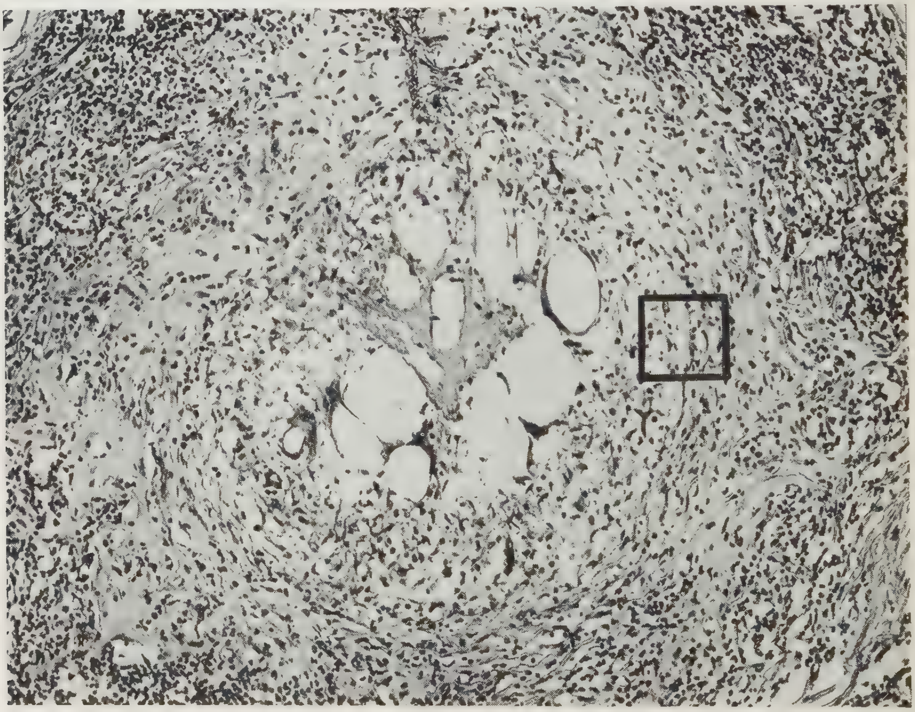


FIG. 5.

Case L.S. Higher magnification (× 100) of single nodule indicated by arrow in Fig. 4.

plantation site removed from patient L.S. who had been implanted 236 days previously with 10 mg of a-estradiol crystals. One can clearly see in this section at least 14 minute discrete, conglomerations of cells, which have the appearance of small tubercles. With higher magnification (Fig. 5), the resemblance of these accumulations of cells to foreign body tubercles becomes even closer.

These tubercles consist of a central core of tissue containing several cavities of variable size, which are delimited by fine connective tissue septa. The latter contain many irregular nuclei which vary considerably in size and shape. Some of the nuclei are giant sized and no separate cell margins can be identified. This central core is surrounded by a zone of hyalinized tissue which contains numerous irregularly shaped cells with large vacuoles and a scattering of foreign body giant cells (Fig. 6).

Surrounding the above described central structure are 3 layers of cells which resemble, in miniature, the 3 layers of cells which make up the gross capsule previously described.

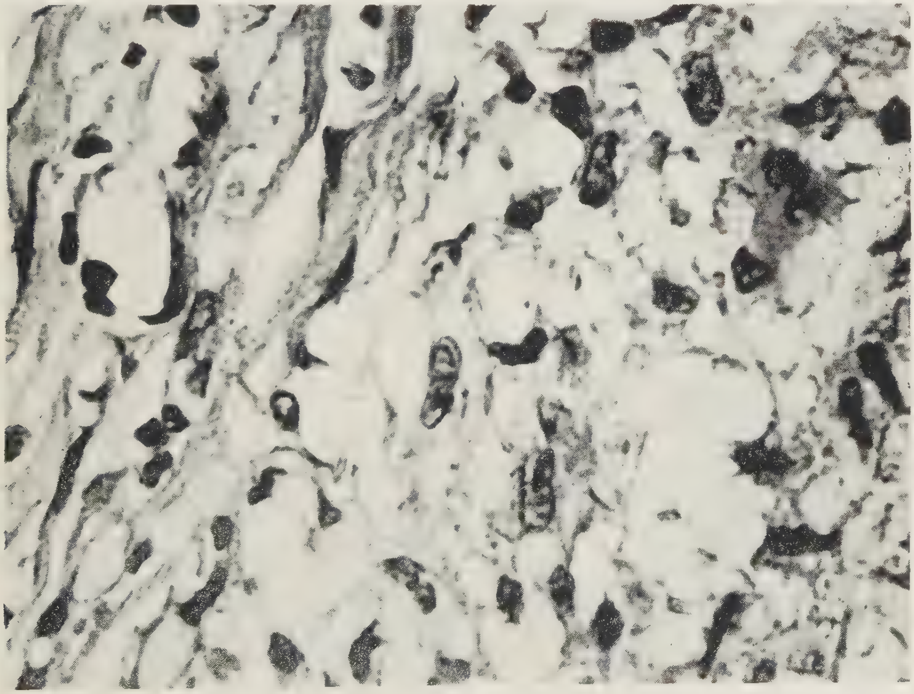


Fig. 6.

Case L.S. High power magnification (× 1200) of boxed-in area of Fig. 5 showing large multi-nucleated vacuolated cells.

Summary and Conclusions.

The local tissue response to implanted crystals and pellets is a typical non-specific foreign body reaction. Following the implantation of crystals of a-estradiol and a-estradiol benzoate, the tissue surrounding the crystals responds by the formation of tiny nodules. In contrast to this type of reaction, a thick avascular capsule forms about the pellets of the implanted hormone. It is important to note that the epidermis overlying the implants showed no evidence of cellular atypism and, similarly, in no instance did the subcutaneous tissues in contact with, and adjacent to, the implanted hormone show any abnormal cellular proliferation.

A significant observation which emerges from this study is the probable effect that the thick avascular capsule has on the absorption rate of the estrogenic substances employed. It was noted that the crystals had a strikingly more prolonged therapeutic and physiologic effect than the pellets composed of the same estrogenic substance and of comparable weight. It would appear, therefore, that the capsule might have a marked retarding effect on the rate of absorption of the hormone. Apparently, with the passage of time, the absorption rate is progressively diminished by the growing thickness of the capsule around the pellet. After a period of approximately 3 months, absorption is either completely stopped or so reduced as to have no demonstrable physiologic or therapeutic effect. It seems, therefore, that for purposes of implantation, pellets of a-estradiol and a-estradiol benzoate are not as efficient as crystals of the same chemical constitution.

11313 P

Portals of Entry of Poliomyelitis Virus in the Chimpanzee.*

HOWARD A. HOWE AND DAVID BODIAN. (Introduced by Carl Hartman.)

From the Department of Anatomy, Johns Hopkins University.

The lack of any direct evidence concerning the portal of entry of poliomyelitis virus in man has led to many animal experiments. The use of the resistant rhesus monkey and of "monkey strains" of virus in this problem, however, is open to the objection that the experimental conditions at the outset are widely divergent from those under which the disease takes place in human beings. A closer approxima-

---

* Supported by a grant from the Commonwealth Fund.

tion to the actual situation of the epidemic may be obtained by the use of human stools as a source of virus and the chimpanzee as a near substitute for man. The chimpanzee was selected for this purpose because of its phylogenetic relationship to man and because of a report by Müller[1] that during an outbreak of poliomyelitis in Cologne, Germany, 2 chimpanzees in a children's zoo apparently contracted the disease by natural contagion. One of these animals subsequently died and an examination of the central nervous system by Spielmeyer revealed lesions characteristic of poliomyelitis.

Five chimpanzees were inoculated with a pool of 7 different untreated human stools each of which had previously been found positive by intranasal inoculation into rhesus monkeys.[2] The results are briefly summarized in Table I. Three animals received large inoculations on 5 successive days by stomach tube, while the other two were given 2 cc of untreated stool intranasally and by mouth, respectively, on 3 successive days. In each instance special care was taken to exclude portals other than the one inoculated, and in the case of the last animals (A105, A106), both olfactory tracts had been sectioned intracranially 4 and 5 days previously. All animals except the first (A48, which received stool by stomach tube) contracted typical flaccid paralysis of the left arm. The animal with cut olfactory tracts which received stool by mouth had an initial right facial paralysis which later extended also to the left arm. In all, sections of the cervical cord showed heavy poliomyelitic lesions. Cord and stools from A83 and A105 reproduced the disease in rhesus monkeys. Despite the typical form of the paralysis and lesions in A71, virus could not be demonstrated in either the cord or stools. The stools and cord of A106 have not been tested for virus as yet.

The olfactory bulbs of A83 (inoculated intranasally) showed maximal perivascular cuffing, neuronophagia and cellular infiltration. In contrast to this the olfactory bulbs of A71, the normal animal receiving stool by stomach tube, showed no signs of virus invasion. There was evidence of a meningitis around the olfactory nerve fila which was in keeping with the generalized meningitis over all exposed pial surfaces of the brain. The bulbs of the animal subjected to bilateral olfactory tract section (A105) showed similar changes. There was a generalized meningitis (the spinal fluid white cell count was 900 per cu mm). There were also slight infiltrations of lympho-

---

[1] Müller, W., *Monatschr. f. Kinderheilk.*, 1935, **63**, 134.

[2] Howe, Howard A., and Bodian, David, *J. Infect. Dis.*, 1940, in press.

TABLE I.

Chimpanzee	Mode of inoculation (untreated stool pool)	Intranasal rhesus controls	Incubation period from day of 1st inoculation	Lesions		Cord passage
				Olf. bulbs	Spinal cord	
A48, *ca.* 5 yrs	Stomach tube, 95 cc stool pool	Prostrate	No paralysis			
A71, " 4½ "	Stomach tube, 250 cc stool pool	"	14 days, lt. arm paralysed	Neg.	4 +	Neg.
A83, " 6-7 "	Intranasal, 6 cc stool pool	"	8 days, lt. arm paralysed	4 +	4 +	Pos.
A105, " 3½ "	Olf. tracts cut, stomach tube, 235 cc stool pool	"	9 days, lt. arm paralysed	Neg.	4 +	Pos.
A106, " 3½ "	Olf. tracts cut, 6 cc stool pool by mouth	"	15 days, rt. face, lt. arm paralysed	Neg.	4 +	

cytes and losses of mitral cells which were probably the result of retrograde changes following the tract sections. The olfactory bulbs of A106 showed no inflammatory infiltrations. The completeness of the olfactory tract section in these cases rules out any possibility of virus having reached the brain by this route.[3] †

The failure of chimpanzee A48 to contract poliomyelitis cannot readily be explained, since 2 intranasal rhesus controls were paralyzed in 10 days. A period of 10 days elapsed between the first and second stomach tube inoculations of 25 cc of stool. This was a relatively small dose as compared with those given by the same method to the other animals. On the sixth day following the first inoculation the animal's temperature rose from 100.4° to 104.6° but on the next day had dropped to its former level. The animal has remained entirely well for 6 months. Whether this fever indicated an abortive attack of poliomyelitis cannot be stated. In contrast with this is the finding that A106, a younger animal, became paralyzed after receiving only 6 cc of stool by mouth. The fact that the initial paralysis was bulbar suggests the oral cavity and pharynx as a portal of lower threshold than the gastrointestinal tract.

Summary. Typical poliomyelitis has been produced in 4 chimpanzees by the intranasal, intragastric, and oral inoculation of untreated human stools. In 2 instances the olfactory portal was ruled out by section of both olfactory tracts.

---

[3] Brodie, M., and Elvidge, A. R., *Science,* 1934, **79**, 235; Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 728; Howe, Howard A., and Ecke, Robert S., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 125.

† Vignec, Paul and Trask's[4] feeding experiments on monkeys unfortunately do not exclude this possibility.

[4] Vignec, A. J., Paul, J. R., and Trask, J. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 246.

11314

Substance in Kidneys and Muscle Eliciting Prolonged Reduction of Blood Pressure in Human and Experimental Hypertension.*

I. H. PAGE, O. M. HELMER, K. G. KOHLSTAEDT, P. J. FOUTS, G. F. KEMPF AND A. C. CORCORAN.

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana.

Evidence published elsewhere[1-8] strongly suggests that an inhibitor to the pressor action of angiotonin and renin is contained in kidneys and probably muscle and lung.

Methods. Extracts prepared from fresh kidneys, lung and muscle were tested for their ability to reduce arterial pressure in dogs made hypertensive by the silk or cellophane perinephritis method.[8] Blood pressure was determined by direct femoral arterial puncture. Hypertensive rats (Drury method[9]) were also employed, the blood pressure being measured in the tail by the method of Williams, Harrison and Grollman.[10] The blood pressure of the animals was stabilized at the elevated pressure before extracts were assayed. Some of the dogs exhibited the syndrome of malignant hypertension. Extracts were given by injection subcutaneously, into muscles or by mouth.

The extracts have been tested also on patients with typical essential hypertension and malignant hypertension after the patients had been studied for long periods in the Lilly Clinic. The studies were conducted much as those described previously in evaluation of the various surgical treatments of hypertension (Page and Heuer[11]).

Several experiments have been performed to ascertain whether the ability of plasma to activate renin has been reduced after administration of extracts. The method employed was that of Page and Helmer.[6]

---

* A preliminary report of this work was made at the American Association for the Advancement of Science, Section N, Columbus, Ohio, December 27, 1939.

[1] Page, I. H., *J. Exp. Med.*, 1939, **70**, 521.

[2] Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.

[3] Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, **19**, 92.

[4] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 29.

[5] Tigerstedt, R., and Bergmann, P. G., *Skand. Arch. Physiol.*, 1898, **8**, 223.

[6] Page, I. H., and Helmer, O. M., *J. Exp. Med.*, 1940, **71**, 495.

[7] Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

[8] Page, I. H., *J. A. M. A.*, 1939, **113**, 2046.

[9] Drury, D. R., *J. Exp. Med.*, 1938, **68**, 693.

[10] Williams, J. R., Jr., Harrison, T. R., and Grollman, A., *J. Clin. Invest.*, 1939, **18**, 373.

[11] Page, I. H., and Heuer, G. J., *Arch. Int. Med.*, 1937, **59**, 245.

Extracts of Kidney and Muscle. Extracts of minced tissues were prepared from pork kidney and from rat, rabbit and beef muscle by 6 general methods of extraction; (a) with saline, (b) with acetone, (c) with alcohol, (d) by fractional precipitation with $(NH_4)_2SO_4$, (e) by isoelectric precipitation of water and saline extracts, and (f) saline and acetic acid followed by heating to 58° C. The materials obtained were concentrated *in vacuo* at low temperatures, and, in most cases, dialyzed for 24 hours or more. The concentrated solutions were passed through Seitz filters before intramuscular administration to human beings. Details of the procedures used and results obtained are shown in the accompanying tables.

Relative Potency of the Various Extracts in Dogs and Rats. The filtrate from the isoelectric precipitation of saline extracts of kidney seemed most effective. The amount of extract equivalent to 300 g of original kidney when injected intramuscularly in dogs, over a period of 2 to 4 days, caused marked reduction in arterial pressure (Figs. 1 and 2). The isoelectric precipitate also contains some of the inhibitor.

TABLE I.
Effect of Tissue Extracts on the Arterial Blood Pressure of Dogs with Hypertension.

Type of extract	Estimated* effectiveness	G of fresh kidney required to produce maximum fall in B.P. from hypertensive (>180 mm Hg) levels
Saline extract of whole kidney	+++	600
Isoelectric precipitate of saline kidney extract	+++	400
Filtrate of an isoelectric precipitate of a saline kidney extract	++++	300
0.3 saturated $(NH_4)_2SO_4$ precipitate of saline kidney extract	++	—
0.3 to 0.8 saturated $(NH_4)_2SO_4$ precipitate of kidney extract	0	—
70% acetone extract of kidney	+++	2100
50 to 70% alcohol extract of kidney	++	6000
Extracts of lung	+	—
Filtrate of heated saline-acetic acid extract	++++	960

*Results given in table are an estimate of the effectiveness of the extracts in reducing blood pressure based on the following grades:

+	Transient effect.
++	Some reduction in blood pressure but not reduced to normal level.
+++	Definite reduction in blood pressure but not sustained.
++++	Marked reduction (reduced to normal level), well sustained.

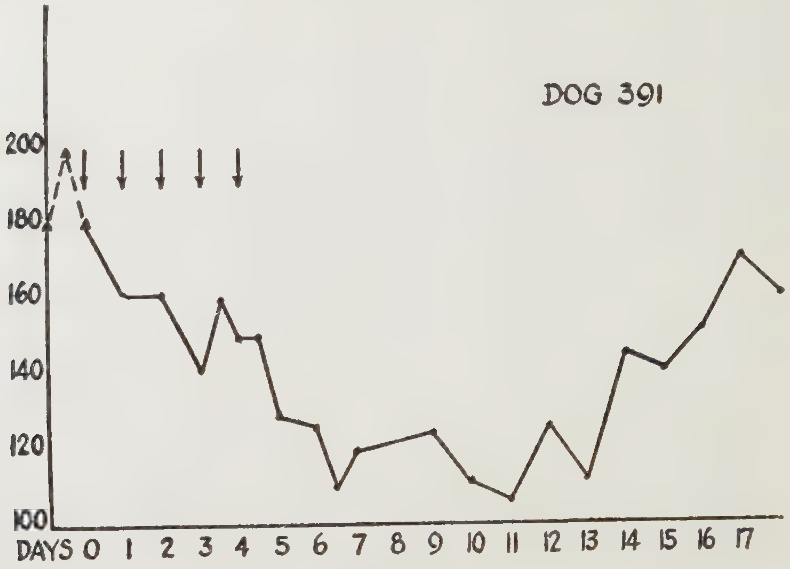


FIG. 1.
Aqueous extract equivalent to 120 grams of beef kidney given to a hypertensive dog.

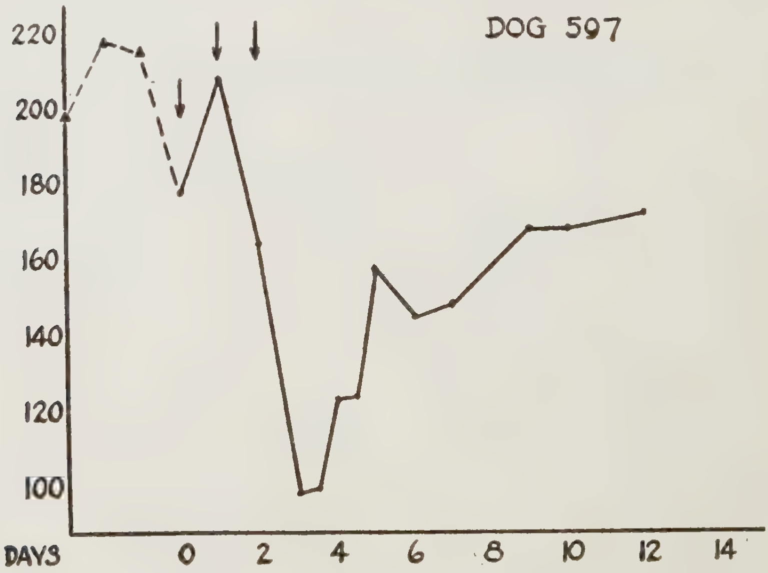
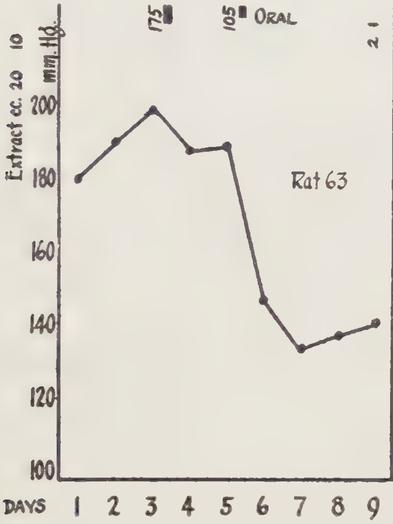


FIG. 2.
Filtrate after isoelectric precipitation equivalent to 100 grams of kidney daily.

The acetone extracts of the kidneys were effective. In only one instance did a 70% acetone extract fail to reduce the dog's pressure when injected intramuscularly. The alcohol extracts were the least effective.

It will be noted from Table II that 70% acetone extracts of muscle, 50% alcohol extracts, and the isoelectric filtrates and redissolved precipitates at pH 4.2 are most active. Poor results were noted with 70% acetone extracts of rabbit muscle, in which an average dose of



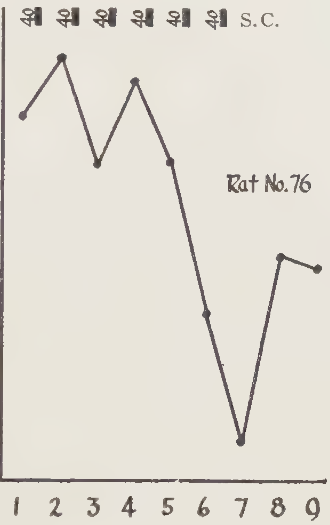


Fig. 3a.

Filtrate given orally after isoelectric precipitation equivalent to 380 grams of beef muscle.

Fig. 3b.

Filtrate given subcutaneously. Same extract as 3a.

TABLE II.

Effect of Muscle Extracts on Arterial Blood Pressure of Rats with Hypertension.

Type of extract	Estimated effectiveness	G of fresh muscle required—Avg
70% acetone	++++	162 (105*)
90% acetone precipitate from 70% extract	+++	190
90% " filtrate " " "	++	220
50% " extract of 70% precipitate	+	380
50% acetone extract	—	360
50% alcohol "	++++	190
70% " "	+++	520
50% alcohol in presence of 10% sodium chloride	—	250
0.3 saturated ammonium sulphate	++	—
0.3-0.5 saturated ammonium sulphate	+	—
0.5-full saturated ammonium sulphate	++	—
Isoelectric precipitate	+++	119
Filtrate of an isoelectric precipitate	++	149
Filtrate of heated saline-acetic acid extract	++++	126

105* Average dose of rat muscle extract. 162 g includes both rat and rabbit.

250 g muscle had little effect, while 105 g of rat muscle seemed quite effective. The data at hand do not establish a similar difference between beef and rat muscle.

Effect of Kidney and Muscle Extracts on Patients. Eight patients have been treated with extracts. In all a sharp prolonged fall in arterial pressure occurred whether the hypertension was of the malignant or essential variety. Symptomatic improvement has been marked. After discontinuing the extracts for 3 to 5 days the blood pressure tends to rise again. What we consider to be a typical curve is illustrated in Fig. 4.

Effect of Kidney Extracts on Renin- and Angiotonin-Activator. Plasma from hypertensive dogs exhibits greater than normal ability to cause vasoconstriction when renin or angiotonin is added to it and the mixture perfused through an isolated rabbit's ear (Table III). When the arterial pressure is reduced by means of kidney extracts, this enhanced ability is reduced or lost.

Discussion. There seems little possibility that the observed prolonged fall in arterial pressure could be due to any of known depressor substances for 3 reasons. First, the fall occurs very slowly—from 12 to 48 hours—depending on dosage. Second, all crystalloidal

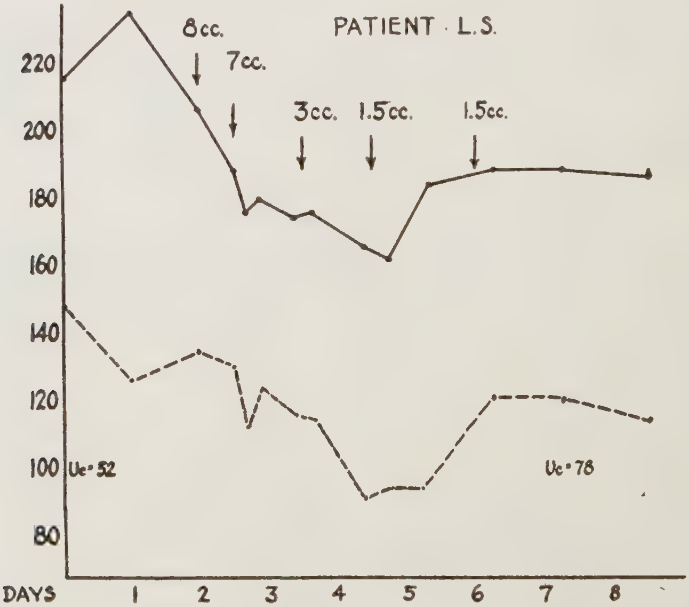


FIG. 4.

Administration subcutaneously of isoelectric filtrate equivalent to 800 grams of kidney to a patient with malignant hypertension. Urea clearance before extract was 52 percent of normal and 7 days later 78 percent of normal.

TABLE III.
Effect of Injection of Kidney Extracts on Activator Content of Hypertensive Dog's Blood.
Dog No. 391—Uninephrectomy plus cellophane on remaining kidney.

Date	Initial drop rate per min.	Substance perfused	Reduction of flow time in min	% reduction of flow	B.P. in mm Hg.	Extract
		cc				
11-20-39	146	.2 plasma of dog No. 391	0	0	184	None
	141	.2 ,, normal dog	0	0		
	140	.2 normal dog plasma + renin	1	39		
	145	.2 No. 391 + renin	4	68		
12-6	131	.2 No. 391	0	0	112	Saline extract of kidney, concentrated
	135	.2 normal plasma	0	0		
	132	.3 No. 391 + renin	½	20		
	135	.3 normal plasma + renin	3	42		
	132	.2 angiotonin + 0.2 cc No. 391	1½	31		
	130	.2 ,, + 0.2 ,, normal plasma	4	61		
12-20	147	.2 normal plasma	0	0	176	None
	146	.2 No. 391	0	0		
	150	.3 normal plasma + renin	1½	42		
	147	.3 No. 391 + renin	5½	81		
	144	.2 angiotonin + 0.2 cc normal plasma	1½	29		
	148	.2 ,, + 0.2 ,, No. 391	4½	64		
1-29-40	95	.1 normal plasma	0	0	52	50% alcohol oral extract
	94	.1 No. 391	0	0		
	91	.1 normal plasma + renin	2	52		
	94	.1 No. 391 + renin	1	20		
	92	.2 No. 391 + 0.2 cc angiotonin	½	12		
	93	.2 normal plasma + 0.2 cc angiotonin	2	39		

substances would have been lost during dialysis, for the inhibitor is non-dialyzable. Third, the reduction in pressure remains for several days in contradistinction to the transient reduction resulting from injection of histamine, choline, adenylic acid, etc.

Injection of kidney and muscle extracts reduce or abolish the ability of the plasma to activate renin and angiotonin. This may be interpreted as either reduction in the amount of activators or increase in the amount of inhibitors.

It has seemed of especial interest that in 4 dogs suffering from the syndrome of malignant hypertension that injection of extracts has reduced their blood pressure and caused improvement in the signs in the eyegrounds. Two human beings with malignant hypertension have also been treated. The fall in arterial pressure has been striking (Fig. 4). Urea clearance in these patients also exhibited a change for the better. It is too early to assert that over prolonged periods these extracts will have therapeutic value. Animal tests suggest that they may have value.

Most of our extracts have been given by injection, but the few that we have tried by mouth in rats have proven effective. Harrison, Grollman and Williams were the first to show that kidney extracts were effective by mouth.†

Summary. Extracts of kidney and muscle effectively reduce arterial pressure in hypertensive human beings, dogs and rats for prolonged periods and simultaneously reduce the ability of plasma to activate renin and angiotonin.

---

† The present results on extracts of kidneys, although arrived at by an entirely independent route of investigation, are in accordance with those of Harrison, Grollman and Williams which are now in press.

11315

Experimental Hypoprothrombinemia with Anti-Prothrombin Serum.

L. M. Tocantins.

From the Division of Hematology, Jefferson Medical College and Hospital, Philadelphia.

A transitory reduction in the plasma prothrombin of rabbits may be produced by injecting into these animals an antiserum prepared in the guinea pig against fibrinogen-free rabbit prothrombin.

Fibrinogen-free prothrombin was isolated from the plasma of rabbits as follows:[1] About 100 cc of blood was collected from a large rabbit by cardiac puncture, in 5 cc of 5% sodium citrate; the plasma was separated off, passed through a Berkefeld V filter and the clear filtrate then heated carefully to 54-56°C for 2 minutes, the precipitated fibrinogen being filtered off and discarded. The plasma was then diluted 10 times with distilled water, and 1% acetic acid was added slowly with constant stirring until a pH of 6.0 was reached. The precipitate that formed was, after centrifugation, diluted in about 5 cc of salt solution, to which a few drops of 0.5% sodium bicarbonate were added. On standing at room temperature, most of the precipitate was dissolved. To prepare the antiserum the prothrombin solution from 100 cc of blood was injected intraperitoneally into a guinea pig weighing no less than 600 g, 5 times, at approximately 6-day intervals, the animal being being killed and bled out 10 days after the last injection. Rabbits weighing between 2.5 and 3.0 kg were used; they were fed a mixture of clover hay, timothy, oats, corn and dog chow. Blood was drawn from the heart or ear vein into a syringe containing 1.34% sodium oxalate solution (0.2 cc of the solution, 1.8 cc of blood). Prothrombin was measured according to the method of Quick.[2] With each determination in the experimental animals, control determinations were performed on plasma from normal rabbits.

Mixing increasing dilutions of the antiserum with a clear prothrombin solution brought about flocculation and precipitation of the prothrombin in dilutions up to 1-256. By incubating the undiluted antiserum with a given amount of oxalated rabbit plasma for 24 hours and following it with the addition of thromboplastin and calcium chloride it was possible to demonstrate a complete inactivation of the prothrombin in that plasma, and some reduction in prothrombin activity in dilutions of the antiserum up to 1-128.

---

[1] Ferguson, J. H., *J. Lab. and Clin. Med.*, 1938, **24**, 273.

[2] Quick, A. J., *Am. J. Physiol.*, 1937, **118**, 260.

Subcutaneous injections into 2 rabbits in doses of 0.2 to 0.4 cc per kg body weight of the animal produced no detectable external changes either locally or generally. In this respect the serum differs from antiplatelet serum which produces large local hemorrhages when injected subcutaneously.[3] In the doses employed, injections of the antiserum intravenously in 4 healthy, nonfasting animals produced only a very transitory reduction in the prothrombin, varying between 25 to 40% of normal, detected usually between 15 and 20 minutes after an injection. There was a quick recovery by the end of the first hour and no subsequent reduction was detected. Repetition of the injection the next day brought about a similar response, followed later by a rise of the prothrombin concentration above normal and failure of subsequent injections to bring about a drop.

In 4 animals that had been fasting for 3 to 4 days it became easier to produce and maintain a hypoprothrombinemia by this method. Chart I illustrates the effects of one or more intravenous injections in 2 of these rabbits deprived of food for 3 days before the antiserum was given and while the experiments were being carried out. Fasting for 3 days in itself produces only a very slight reduction in the plasma prothrombin. Two animals died during the experiments from hemorrhage induced by cardiac punctures. When size of the veins made it possible all specimens in one animal were collected from the ear vein. Selection of animals with large ear veins makes it possible to collect rapidly the required amount of blood.

The rapidity with which the prothrombin returned to normal after being reduced by the injection of antiprothrombin serum is another indication of the great lability of this substance. The body reserves of vitamin K and of whatever other substances are required for the production of prothrombin by the liver seem to make a preliminary period of fasting necessary to demonstrate a clear-cut diminution of the prothrombin after injection of the antiserum.

The fact that some of the prothrombin in the plasma may be associated with other protein fractions besides the globulin may partially explain the moderate and transitory nature of the reduction in the plasma prothrombin; the antiserum may only destroy that portion of the prothrombin linked with the globulin fraction. Heating of the plasma to precipitate the fibrinogen may have also precipitated or destroyed some of the prothrombin and affected the antigenic properties of this protein. In no instance was the plasma prothrombin

---

[3] Tocantins, L. M., *Arch. Path.*, 1936, **21**, 69.

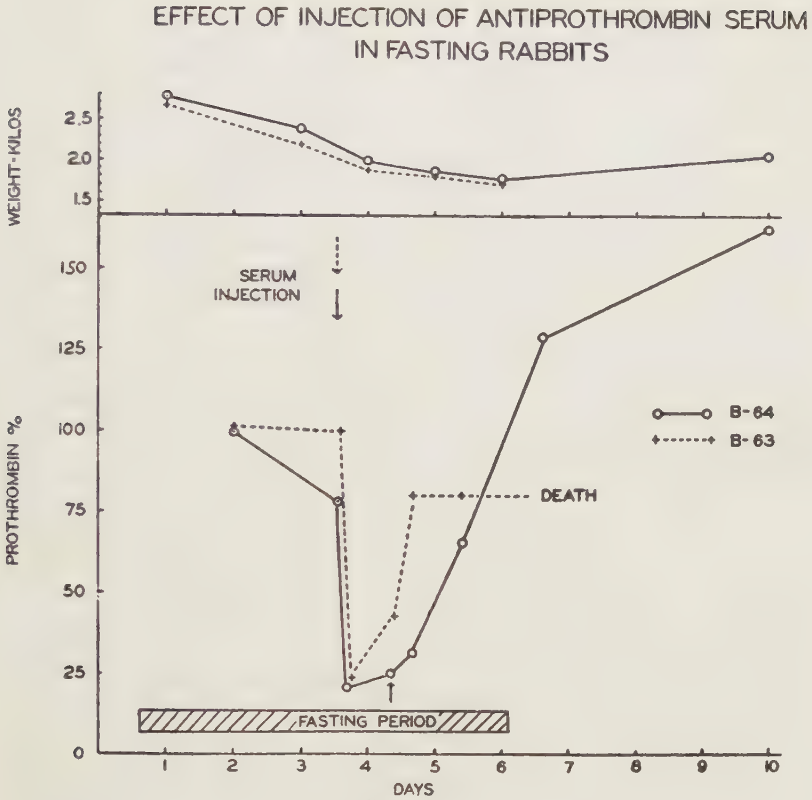


Chart 1.

Rabbit B-64 received 2 injections of the antiserum (solid arrow) and survived the experiment. Rabbit B-63 received 1 injection only (broken arrow) and died on the following day from a hemopericardium.

ever observed to go below 20% of normal; this may explain the relative scarcity of hemorrhagic manifestations encountered.

Summary. An antiprothrombin serum may be prepared in the guinea pig by injecting it with fibrinogen-free prothrombin isolated from rabbit plasma. Intravenous injections of this antiserum in fasting rabbits produce a hypoprothrombinemia of a transient and moderate character.

11316

Effect of Thymectomy in Immature Rats.*

William O. Reinhardt. (Introduced by Herbert M. Evans.)

From the Department of Anatomy and the Institute of Experimental Biology, University of California, Berkeley, California.

It has been reported[1, 2] that Roentgen irradiation of the thymus of 2-day-old rats results in the temporary arrest of development of the spermatogenic tissue of the testis. An analysis of recent literature shows that surgical thymectomy has been carried out on the following age groups of rats: female, age 1 day and male and female, age 21 days;[3, 4] male and female, age 10-23 days;[5] male, age 25 days,[6] and male and female, age 21 days.[7] In none of these experiments were significant effects noted on growth, development, age of puberty, fertility, or differentiation of the endocrine organs.

With the above reports in mind and in order to explain if possible the difference between surgical and Roentgen ray thymectomy in immature animals, the following experiment was carried out.

Thymectomy was performed on half the animals of 10 litters of the Long-Evans strain of rats. Nine litters were operated upon at 4 days of age and one litter at 2 days of age. A total of 46 animals were employed of which 30 were males and 16 were females. The operated animals, both thymectomized and sham operations, were distributed according to littermate controls of equal body weight and size. Thymectomy was performed under ether anesthesia using a midline neck incision involving splitting of the upper part of the sternum, the thymus gland being readily exposed, and removed en masse by blunt dissection. Postoperative complications were negligible and the animals recovered from the effects of the operation without harm. Five litters (20 animals) were autopsied at 33 days

---

* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation.

[1] Shay, H., Gershon-Cohen, J., Fels, S., Meranze, D., and Meranze, T., *J. Am. Med. Assn.*, 1939, **112**, 290.

[2] Gershon-Cohen, J., *et al.*, *Science*, 1938, **87**, 20.

[3] Andersen, D. H., *J. Physiol.*, 1932, **74**, 49.

[4] Andersen, D. H., *J. Physiol.*, 1932, **74**, 207.

[5] Pappenheimer, A. M., *J. Exp. Med.*, 1914, **19**, 319.

[6] Hashimoto, E. I., and Freudenberger, C. B., *J. Am. Med. Assn.*, 1939, **112**, 1680.

[7] Segaloff, A., and Nelson, W. O., *Anat. Rec.*, 1940, **76** (2), Supplement No. 2, 50.

of age and 5 litters (26 animals) at the age of 87 days. Daily observations were made of the gross behavior of the animals and weekly weights were determined. At autopsy, the weight of the pituitary, gonads, adrenals, thyroid, spleen and kidneys was taken and compared with the body weight. The neck region was thoroughly examined at autopsy and all suspicious tissue subjected to microscopic examination of stained sections. All tissues were routinely examined microscopically using hemotoxylin and eosin stained celloidin sections.

Daily observation of the thymectomized and control animals maintained under the same experimental conditions (diet and handling) demonstrated no apparent difference in the gross behavior, size, time of opening of the eyes, eruption of the incisors and ears, descent of the testes, opening of the vagina, or character of the hair. Body weights and growth curves were of the same character. At autopsy, and after microscopic examination, the neck region of 2 of the 29 thymectomized animals was found to contain small amounts of thymic tissue (estimated at about 1/7th of the normal). Histological examination of the tissues removed at autopsy revealed no apparent difference between thymectomized and controls. There was no arrest of spermatogenesis in the testes of either age group as compared with the controls.

Conclusions. It is felt that on the basis of the reports quoted and on the above data, thymectomy by surgical methods has no apparent effect on the growth, development, or endocrine organ differentiation of the rat when performed at the age of 1-2 days and studied past the onset of puberty. Since surgical thymectomy results in no apparent effects as studied above, it is probable that irradiation thymectomy can have no further effect, and that the cause of the effects described[1, 2] must be sought elsewhere.

11317

Decorticate and Decerebrate Preparations Produced by Insulin Shock.*

EUGENE ZISKIND AND DAVID B. TYLER. (Introduced by C. H. Thienes.)

From the University of Southern California, School of Medicine, Los Angeles.

During insulin shock induced therapeutically there is a progressive depression of nervous functions, in a gradient manner, from the highest (cortical) levels to lower subcortical levels (striatum, midbrain, medullary) which is temporary and reversible.[1, 2] It was decided to demonstrate this gradient effect clinically with the production of animal preparations showing permanent, irreversible lesions at progressively lower levels in the nervous system by prolonging the period of hypoglycemia.

Cats, fasted 18 hours, were given 15 to 20 units of insulin per kilo body weight. When clinical signs indicated early medullary decompensation, small amounts of glucose were given intraperitoneally to prevent death and still maintain a marked degree of hypoglycemia. Hypoglycemia was terminated after 12 to 20 hours. Persistent brain damage was observed in 17 animals. Group I consisted of 5 animals with variable degrees of cortical damage. Group 2 totaled 8 animals with cortical plus subcortical injury ("thalamic preparations"?). Group 3 included 4 cats with loss of function at the midbrain level or lower.

The decorticate preparations (Group 1) showed impairment of vision, impairment of placing and hopping reactions (Bard), slight impairment of righting reactions, difficulty in feeding self, impairment of cleaning habits, restlessness and absence of rage reaction when confronted by a dog. They were able to walk and maintained normal body temperature. These preparations were kept alive from 6 to 90 days.

Preparations in Group 2 showed in addition to the above, inability to walk, decerebrate rigidity, increased tonic neck reflexes and mock rage. These animals were also able to maintain body temperature. These signs point to loss of function at the "thalamic level." The

---

* The authors are indebted to Eli Lilly Company for the insulin utilized in this study.

[1] Angyal, L. V., *Z. f. d. ges. Neurol. u. Psychiat.*, 1937, **157**, 35.

[2] Frostig, J. P., *Arch. Neurol. and Psychiat.*, 1938, **39**, 219; also table in *Am. J. Psychiat.*, 1939, **96**, 373.

animals lived 19 to 48 hours after the hypoglycemia was terminated.

Animals in Group 3 were not studied as carefully as the above, but are added because they showed inability to maintain body temperature. They remained in a state of coma until death which occurred from 10 to 18 hours after the interruption of the hypoglycemia. Because of the poikilothermia it is assumed that the damage is at the midbrain level or lower.

Finding clinical evidence of damage at a certain level in the nervous system does not also establish the contention of simultaneous injury of the higher centers. This assumption appears warranted at this time from the clinical course of the animals under the experiment. During the induction of hypoglycemia, the gradient depression of level after level of nervous functions is easily followed and on termination of the hypoglycemia the reversal of this order is obtained. Histopathologic studies are in preparation to establish the proof for this contention.

The demonstration of a gradient effect by insulin in the central nervous system may have a very significant bearing on insulin shock treatment. The effects of this therapy are directed towards psychotic symptoms presumably arising in the highest cerebral centers. Therapeutic effects of the same type now obtained may occur with milder but more prolonged degrees of hypoglycemia, still capable of affecting the highest centers but sparing the lower medullary centers. This would remove the present hazards of shock therapy. This will be subjected to clinical verification.

The technic involved in this study may have an advantage over operative transection or the vascular decerebration of Pollack and Davis for studying the different physiologic strata of the central nervous system. It may be that with insulin the cleavage is along more definite physiological lines than is possible with these other "anatomic" methods.

Our cordial appreciation is expressed to Dr. Clinton H. Thienes of the department of pharmacology and to Dr. Douglas R. Drury of the department of physiology, for both facilities and many helpful suggestions.

11318 P

Preparation and Properties of Soluble Bacterial Enzymes Which Decompose Creatinine.

M. J. Carl Allinson. (Introduced by H. H. Beard.)

From the Louisiana State University School of Medicine, New Orleans.

Several years ago, soil bacteria capable of producing adaptive enzymes which specifically decompose creatine and creatinine were isolated.[1, 2] This report presents preliminary observations on the manner of preparation and properties of soluble enzymes prepared from one species (NC) of these bacteria.

When the washed cells are desiccated by various methods a portion of the enzymic system which decomposes creatinine is rendered soluble. This soluble portion can be obtained by adding water to the dried suspension, centrifuging, and collecting the clear supernatant solution. The methods that have been used include grinding with abrasive materials, such as pumice-stone, desiccation with phosphorus pentoxide and sulphuric acid, and desiccation with alcohol-ether, or acetone-ether.

The method of assay employs 0.5 mg of neutral creatinine-hydrochloride as substrate, 0.5 cc of 0.5 M phosphate buffer pH 7.0, the substance being tested, and water to 5 cc. The suspension is incubated at 38° to 40°C for 30 minutes. The undecomposed creatinine is determined colorimetrically after the addition of alkaline picrate.

The activity of the preparation obtained under the above conditions represents about 10% of the activity of the original cells when assayed under the same conditions. The properties of the extract differ in certain respects from the original cells. Sodium cyanide 10^{-3}M, and thymol inhibit the cells but not the extract. Toluene and formaldehyde depress the activity of the cells and extract. Sodium fluoride, 10^{-3}M, does not inhibit the cells or extract.

Studies on the purification of the enzymes are in progress.

---

[1] Miller, B. F., and Dubos, R. J., Proc. Soc. Exp. Biol. and Med., 1936, **35**, 335.

[2] Dubos, R. J., and Miller, B. F., *J. Biol. Chem.*, 1937, **121**, 429.

11319 P

Effect of Testosterone Propionate Upon Thyroid and Parathyroid Glands of Intact Immature Female Rat.*

IRA T. NATHANSON, AUSTIN M. BRUES AND RULON W. RAWSON.
(Introduced by Joseph C. Aub.)

From the Laboratories of the Collis P. Huntington Memorial Hospital, Harvard University.

In previous communications it was shown that testosterone propionate acts directly upon the vagina and uterus of the immature female rat.[1] It was also determined that it stimulates the ovary indirectly by way of the anterior hypophysis[1] even in the absence of the adrenals.[2] It is the purpose of this paper to record the effect of testosterone propionate upon the histological appearance of the thyroid and parathyroid gland of the intact immature female rat.

Method of Experiment. Twenty-one immature rats of the Wistar strain weighing between 40 and 48 g and approximately 4½ weeks of age, were divided into 2 groups of 12 and 9 respectively. Five mg of testosterone propionate† in 0.2 cc of sesame oil were given in one dose to each of the first group (12) and 0.2 cc of sesame oil without the hormone were given to the second group (9) to serve as controls.

Group I (Testosterone treated group). Twelve hours after giving testosterone, colchicine (0.05 mg per 50 g of body weight) was given subcutaneously to one animal. This animal was sacrificed 12 hours later (24 hours after testosterone). Thereafter one animal was sacrificed every 24 hours, one dose of colchicine having been given 12 hours previously.

Group II (Controls). Three animals each were sacrificed, 72, 144 and 288 hours respectively after the onset of the experiment. Two of each group of 3 were given colchicine 12 hours before. The remaining animals received no other treatment.

---

* This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

[1] Nathanson, I. T., Franseen, C. C., and Sweeney, A. R., Jr., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 385.

[2] Nathanson, I. T., and Rawson, R. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 482.

† We are very grateful for the liberal quantities of testosterone proprionate which were furnished us by Doctors Gregory Stragnell and Max Gilbert of the Schering Corporation, and by Dr. Ernst Oppenheimer of Ciba Co., under the trade names of Oreton and Perandren respectively.

The thyroid and parathyroid glands of each animal were fixed in Zenker's solution imbedded in paraffin, and representative sections were stained with phloxine methylene blue.

Measurements of height of the acinar cells of the thyroid were made according to the method of Rawson and Starr,[3] in which 100 cells were measured in each gland. Mitoses were counted, using the technic of Brues and Marble.[4] At least 4000 cells were counted in each gland, and at least one entire section of each gland was examined.

Results. Table I presents the results of this experiment.

It can be seen from the results presented in the table that testosterone propionate is capable of stimulating the thyroid and parathyroid glands of the intact immature female rat. The increase in mitotic activity especially in the first 96 hours seems particularly significant. This corresponds very closely with changes seen in the ovary during the same period of time. Increase in mitotic activity was noted even in the first 24 hours. After 96 hours the activity decreased, but in all instances the levels remained at the upper limits, or above those of the controls. In one animal (No. 109), which showed a high degree of activity, corpora lutea were noted in the ovaries. These were not seen in the ovaries of the other animals.

Increase in cell height could be demonstrated in the thyroid gland almost as soon as the increase in mitotic activity was noted. It can be seen that in the early stages of the experiment an increase in mitosis was associated with an increase in the size of the cell. However, there was no direct correlation between the number of mitoses and cell height.

In the control group no marked difference could be detected between the animals treated with colchicine and those that had none, although the mean cell height seemed to be slightly lower in the colchicine-treated group. One control animal (No. 110) deserves comment. In this rat there was an increase in mitosis and cell height in the thyroid, as well as stimulation of the follicles of the ovaries. Autopsy revealed a cyst of the pituitary gland.

It is noteworthy that in all groups mitotic activity in the thyroid and in the parathyroid gland of each animal was approximately equal.

The mechanism of this stimulation is not clear at present. Several possibilities seem tenable.

(a) Testosterone stimulates the thyroid and parathyroid glands directly.

---

[3] Rawson, R. W., and Starr, P., *Arch. Int. Med.*, 1938, **61**, 726.

[4] Brues, A. M., and Marble, B. B., *J. Exp. Med.*, 1937, **65**, 15.

TABLE I.

Effect of Testosterone on Mitosis and Cell Height of Thyroid Gland.

No.	Wt. onset experiment	Wt. when sacrificed	Age when sacrificed in days	Hr after testosterone	Mitoses thyroid per 10,000 cells	Mitoses parathyroid per 10,000 cells	Mean acinar cell height per 100 acini	Remarks
				Treated Group				
114	48	49	32	24	46	28	4.24 ± 0.004	Colchicine
103	46.5	48.5	33	48	41	—	3.82 ± 0.004	,,
115	46	47	34	72	22	33	4.22 ± 0.004	,,
105	45	57	35	96	118	77	4.19 ± 0.005	,,
111	44	59.5	36	120	12	—	4.27 ± 0.004	,,
101	43.5	63.5	37	144	12	10	4.15 ± 0.004	,,
102	43.5	61	38	168	11	22	3.06 ± 0.004	,,
109	43.5	69	40	216	75	—	4.29 ± 0.004	,, corpora lutea
104	42	69	41	240	19	—	4.20 ± 0.004	,,
107	42	85	42	264	18	15	4.05 ± 0.004	,,
113	43	88	43	288	19	22	4.34 ± 0.004	,,
				Control Group.				
116	40	52	35	—	8	11	3.85 ± 0.005	No colchicine
117	45	53	35	—	2	—	3.61 ± 0.004	Colchicine
110	46.5	54	34	—	36	—	4.17 ± 0.005	,, cyst pituitary
108	45.5	68	38	—	6	—	3.58 ± 0.004	Colchicine
118	42	67	39	—	7	—	3.64 ± 0.004	,,
119	41	66	39	—	2	9	3.74 ± 0.004	No colchicine
112	42	72	43	—	7	8	3.66 ± 0.004	Colchicine
120	40	82	44	—	6	4	4.03 ± 0.005	,,
121	42	85	44	—	4	10	3.74 ± 0.004	No colchicine

(b) Testosterone stimulates the thyroid and parathyroid glands either by way of the anterior pituitary or through some other gland of internal secretion.

The second thesis seems more plausible, especially since the changes noted so closely parallel those in the ovary which we have shown are stimulated by way of the hypophysis. Experiments are under way at present to clarify these points.

Conclusion. Testosterone propionate is capable of stimulating the thyroid and parathyroid glands of the intact immature female rat. This stimulation is manifested by increased mitotic activity and by histological evidence of functional activity.

11320 P

The Mechanism of Action of Heparin*

MORRIS ZIFF AND ERWIN CHARGAFF.

From the Departments of Biochemistry and Surgery, College of Physicians and Surgeons, Columbia University, New York.

It is known that heparin does not prevent the coagulation of a fibrinogen solution by purified thrombin.[1] It likewise is unable to prevent the conversion of isolated prothrombin to thrombin.[2, 3] To exert its powerful anticoagulant action, heparin requires an additional factor which is known to be present in blood, plasma, and serum.[1, 4, 5] This factor has been shown by Quick[4] to be contained in the serum albumin fraction.

We have investigated the activity of various components of the albumin fraction in producing the anticoagulant effect of heparin on the fibrinogen-thrombin system.

The *thrombin* was obtained by the method of Eagle,[6] and the fibrinogen was prepared in the usual manner by repeated precipitations with sodium chloride. *Heparin* was used in the form of the pure sodium salt.†

---

* This work has been supported by a grant from the John and Mary R. Markle Foundation.

[1] Howell, W. H., and Holt, E., *Am. J. Physiol.*, 1918-1919, **47**, 328.

[2] Mellanby, J., *Proc. Roy. Soc. (London)*, 1934, B **116**, 1.

[3] Quick, A. J., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 391.

[4] Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

[5] Brinkhous, K. M., Smith, H. P., Warner, E. D., and Seegers, W. H., *Am. J. Physiol.*, 1939, **125**, 683.

[6] Eagle, H., *J. Gen. Physiol.*, 1935, **18**, 531.

The activities of the albumin components studied are shown in Table I, which reproduces a typical experiment. The letters in the first column of this table stand for the following compounds: *A*, crystalline albumin from human serum, prepared by Dr. F. E. Kendall of the Research Division for Chronic Diseases, Welfare Island. We are greatly indebted to Dr. Kendall for different specimens of this substance. *B*, albumin from sheep plasma, prepared according to Howe.[7] *C*, fraction from human plasma insoluble at 55% saturation with ammonium sulfate, presumably containing the globoglycoid of Hewitt.[8] *D*, fraction from human plasma insoluble at 75% saturation with ammonium sulfate. *E*, fraction from human plasma insoluble at 100% saturation with ammonium sulfate. This fraction presumably contained the seroglycoid of Hewitt.[9] *F*, the supernatant solution after removal of E, dialyzed and concentrated by ultrafiltration.

All substances were dissolved in physiological saline and carefully adjusted to the neutral point. The pH measurements were performed by means of the glass electrode. The experiments were carried out at room temperature as follows: 0.1 cc of a thrombin solution (from human plasma), 0.06 cc of a 0.3% heparin solution, and 0.06 cc of the protein solution were mixed. Three minutes later

TABLE I.
Influence of Albumin Fractions on the Anticoagulant Effect of Heparin.

	30″	3′	5′	10′	15′	20′	25′
A, (9 mg N/cc) without heparin	+						
” ” with ”	+						
A, (4.5 mg N/cc) without heparin	+						
” ” with ”	+						
B, (1.4 mg N/cc) without heparin	—	-	+				
” ” with ”	—	—	—	—	—	—	—
C, (1.2 mg N/cc) without heparin	+						
” ” with ”	+						
D, (2.7 mg N/cc) without heparin	—	—	—	+			
” ” with ”	—	—	—	—	—	—	—
E, (1.2 mg N/cc) without heparin	+						
” ” with ”	—	—	—	—	—	—	—
F, (2.2 mg N/cc) without heparin	—	+					
” ” with ”	—	—	—	—	—	—	—

+ = clot.

---

† We wish to thank Hoffmann-LaRoche, Inc., Nutley, N. J., for the heparin preparation used.

[7] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93.

[8] Hewitt, L. F., *Biochem. J.*, 1938, **32**, 26.

[9] Hewitt, L. F., *Biochem. J.*, 1936, **30**, 2229; 1937, **31**, 360.

0.2 cc of a fibrinogen solution was added to the mixture. Fibrinogen preparations from human and sheep plasma gave essentially the same results. In the control experiments 0.06 cc of saline were substituted for the heparin. The tubes were examined for clots at fixed intervals in order to avoid unnecessary agitation. The concentration of the protein solutions used in the experiments reproduced in Table I is expressed in mg N per cc of protein solution.

As shown in Table I, the crystalline albumin fraction even when tested in fresh solution is entirely inactive as a complement of heparin. The activity appears to reside in the most soluble fraction of the serum albumin. It might be mentioned that the albumin solutions prepared by Howe's method retained their activity for more than 50 days. It is not possible to state definitely whether the activity is due to a single component of the albumin fraction. Additional work will have to be carried out with respect to this question. A detailed report on this work and related aspects will be published at a later date.

It might be mentioned that occasionally individuals are encountered whose clotting time responds to heparin to a slight degree only. It will be of interest to see whether the heparin complement here discussed is lacking in the serum of these patients.

11321

Chloride Excretion in Hypothyroidism.

D. J. STEPHENS. (Introduced by W. S. McCann.)

From the Department of Medicine, University of Rochester School of Medicine, and the Medical Clinic, Strong Memorial and Rochester Municipal Hospitals, Rochester, N. Y.

Cutler, Power and Wilder,[1] using a standardized sodium chloride depletion test, have shown that the chloride concentration in the urine of patients with Addison's disease is significantly and consistently higher than in controls. These observations have been confirmed by others.[2] Six of 8 patients with clinical hypopituitarism, studied under similar conditions, have shown increased concentrations of chloride in the urine comparable to those found in patients with

---

[1] Cutler, H. H., Power, M. H., and Wilder, R. M., *J. Am. Med. Assn.*, 1938, **111**, 117.

[2] Dryerre, H. W., *Edinburgh Med. J.*, 1939, **46**, 267.

Addison's disease. Symptoms suggesting those of the Addisonian crisis developed in 4 of the 6 patients with abnormal chloride excretion, and were promptly relieved by the administration of sodium chloride and adrenal cortex extract. These observations were interpreted as evidence of the occurrence of chronic adrenocortical insufficiency in clinical hypopituitarism, presumably secondary to withdrawal of the adrenotropic anterior pituitary principle.[3]

Decourt[4] has recently reported lowered blood chloride levels in myxoedema and suggests that the thyroid gland may play a rôle in the regulation of chloride metabolism. Lowering of the basal metabolic rate is a prominent characteristic of hypopituitarism, and is occasionally observed in patients with Addison's disease. However, in such patients, the administration of thyroid substance for the purpose of raising the metabolic rate may precipitate the crisis of acute adrenal insufficiency.[5, 6] Similar reactions have not been reported in myxoedema. In the present study of a group of patients with primary hypothyroidism, normal values for serum chloride were found and a normal response to sodium chloride restriction was observed.

Six patients with the clinical and laboratory characteristics of primary hypothyroidism were studied. In 2 patients the hypothyroidism was post-operative. In each instance there was a satisfac-

TABLE I.
Chloride Excretion in Hypothyroidism.

Patient	BMR	Urine chloride (as NaCl) 4-hr spec., 3d day. Mg per 100 cc
E.E.	—24	50
M.I.	—24	95
A.T.	—25	58
M.H.	—27	79
D.B.	—30	140
M.K.	—42	49
Controls, 17 cases		111 35-244
Addison's disease, 8 cases*		487 382-593
Hypopituitarism 6 cases		518 364-690
2 cases		65, 240

*Includes cases of Cutler, Power and Wilder.[1]

---

[3] Stephens, D. J., *Am. J. Med. Sci.*, 1940, **199**, 67.

[4] Decourt, J., *Ann. d. med.*, 1938, **44**, 133.

[5] Means, J. H., *The Thyroid and Its Diseases*, J. B. Lippincott Co., Philadelphia, 1937, p. 530.

[6] Lerman, J., and Salter, W. T., *Endocrinology*, 1939, **25**, 712.

tory response to the subsequent administration of thyroid substance. The standard procedure described by Cutler, Power and Wilder[1] was used. The chloride concentration of a 4-hour urine specimen collected on the third day of chloride restriction in each of the 6 patients is shown in Table I. In each case, this value falls well within the range which has been found in control subjects. No significant change was observed in the serum concentration of chloride, the carbon dioxide combining power or the blood non-protein nitrogen. None of the patients experienced any untoward symptoms during the period of sodium chloride restriction.

Conclusions. Six patients with primary hypothyroidism, in whom chloride excretion was studied during periods of sodium chloride restriction, showed no evidences of limitation of adrenocortical function.

11322

Dissociation of Pneumococcus by Radon Irradiation.

T. T'UNG AND CHIEN-LIANG HSÜ. (Introduced by S. H. Zia.)

From the Departments of Bacteriology and Immunology and Radiology, Peiping Union Medical College, Peiping.

Although extensive studies have been made on the bactericidal action of various rays emanated from radio-active substances[1] very little attention has been paid to the cultural and morphological changes that may follow such irradiation. Spencer[2] first called attention to the possibility of producing in a certain number of instances, distinct and stable morphological variants by exposing *Streptococcus hæmolyticus* and *E. typhi* to the *gamma* rays of radium. While some changes were noted in the cultural characteristics of these variants, few details were given. It has occurred to us that in addition to the morphological changes observed by Spencer, variations in cultural characteristics, serological specificity, and virulence might also result from irradiation. A comparison with another dissociant obtainable by the conventional method seems desirable. For these purposes, we have chosen the pneumococcus in our studies as the dissociants of pneumococcus are well known and readily obtainable. In the present communication, the effect of non-lethal *gamma* rays from radon tubes on Type I pneumococcus is described, and the dissociants thus obtained are compared with those produced by immune serum alone.

---

[1] Spencer, R. R., *Public Health Report* (Wash., D. C.), 1934, **49**, 183.

[2] Spencer, R. R., *Ibid.*, 1935, **50**, 1642.

A virulent culture was plated and 10 colonies were picked in order to allow for the possible presence of some "radon-resistant" forms in the original culture. The organisms were grown in test tube 5.5 x 1.5 cm containing 2 cc liver-digest broth containing 1% glucose. After inoculation, radon tubes or bulbs of about 150 millicurie strength, encased in a brass capsule one mm in thickness, 6 mm in diameter and 30 mm in length, was placed in the same tube. In this way, *gamma* rays only were obtained. The tube was then incubated at 37°C. Every 48 hours, the culture was transferred into a new medium with the brass capsule containing radon tubes or bulbs. Every 24 hours the radon in tubes or bulbs was replenished to the initial strength of about 150 millicurie. Plates were made every day and colonies carefully studied. Occasionally, the culture was found to be contaminated, then the transfer was continued by picking some colonies from the plate made from the last irradiated culture. At the same time the following parallel experiments were run: (1) Culture with brass capsule without radon (control), (2) Culture grown in broth containing 5% homologous immune serum, and (3) Culture grown in broth containing 5% homologous serum and irradiated with radon at the same time.

Results. As the results with 2 series of experiments were essentially identical, only one of them is described in detail. After the 2nd transfer, colonies of the irradiated culture were found to be larger and more convex and mucoid than the controls, but returned to normal after another 3 transfers. From then until the 10th transfer, only smooth colonies were obtained. Five of such from the 10th transfer were inoculated into broth and the organisms were found to be fully virulent. Suddenly after one more transfer, striking changes were brought about: the colonies were all small, dry and extremely rough, but were completely soluble in bile and agglutinable by Types 2 and 3 sera. Twenty out of about 200 colonies obtained from the 11th irradiated culture were transferred to broth and tested for virulence. It was found that the sedimented organisms derived from as much as 20 cc of broth culture failed to kill white mice. The surviving animals were susceptible to the virulent organisms. These dissociants remained stable after weekly transfers for at least 6 months. At no time was a smooth colony observed in any of the subcultures. Attempts to revert 10 such colonies by the mouse-method[3] were negative. It thus appeared that the dissociation was complete. Similar results were obtained with the second series, the only difference being that this sudden change in cultural characteristics and virulence took place after the 13th transfer.

The result from the control experiment was as follows: No change was observed with cultures containing brass capsule only, thus ruling out the possible dissociative effect of brass. The cultures grown in 5% immune serum broth also dissociated into rough forms. The following differences, however, were noted: (1) The pneumococcus grown in immune serum showed gradual changes from S to R while that seeded in the presence of radon irradiation exhibited sudden changes. (2) The loss of virulence of the serum-treated organisms appeared less complete. Even after the 20th transfer, 2-3 cc of broth culture could kill mice from which fully virulent pneumococcus could be recovered. In the presence of immune serum, a fewer number of transfers and a shorter period of irradiation were required to bring about dissociation by radiation. Thus in one experiment, only 5 daily transfers were necessary to produce 95% rough colonies from the radiated cultures. They possessed all the characteristics of those produced by radon alone.

From the above observations, it seems that irradiation with *gamma* rays obtained from radon can regularly induce a change of smooth to rough form of pneumococcus so complete that it was impossible to revert the dissociants to the primary state. Furthermore, by a combined action of immune serum and irradiation, it has been possible to achieve the same results but in a much shorter period of irradiation. This latter observation may offer a plausible explanation of the mode of therapeutic action of X-rays in cases of pneumococcal lobar pneumonia.[4]

Summary. Type I pneumococcus could be dissociated by *gamma* ray radiation. The rough forms appeared suddenly, becoming totally avirulent and irreversible. This dissociative change could be achieved in a shorter period of irradiation by the addition of immune serum.

---

[3] Griffith, F., *J. Hyg.*, 1928, **27**, 113.

[4] Scott, W. R., *Radiology*, 1939, **33**, 331.

11323

Recovery of Antigen from Type I Pneumococcus Immune Precipitate.

SZU-CHIH LIU AND HSIEN WU.

From the Department of Biochemistry, Peiping Union Medical College, Peiping, China.

In previous reports[1, 2] from this laboratory, it has been shown that the liberation of antibody from immune precipitate by the action of acid or alkali is due to a shift of equilibrium as follows:

$$B_mG_n \underset{\text{neutral}}{\overset{\text{acid or alkali}}{\rightleftarrows}} B_{m-x}G_n \downarrow + xB$$

Where G = antigen, B = antibody and B_mG_n = immune precipitate formed in neutral solution. Upon treatment with acid or alkali, a part of the antibody, xB, is set free from the precipitate, B_mG_n, leaving behind an acid or alkaline precipitate, $B_{m-x}G_n$. Since the acid or alkaline precipitate, $B_{m-x}G_n$ has a higher antigen content than the neutral precipitate, B_mG_n, it should, when separated from the free antibody, xB, liberate some antigen upon neutralization. The purpose of the present study is to test this point.

Portions of Type I Pneumococcus horse immune precipitate (4 mg N each) were treated with dilute HCl or NaOH of different concentrations in the presence of 1% NaCl according to the technic reported in a previous communication.[2] After centrifuging, the supernatants were used for pH determinations. The acid or alkaline precipitates were evenly suspended in 2 cc of normal saline and neutralized. The neutralized suspensions were centrifuged. One cc portions of the neutralized supernatants were mixed with 1 cc of the same homologous antiserum, which was used in preparing the original immune precipitate, while the remaining portions were mixed with normal serum as control. The mixtures were incubated at 37°C for 2 hours and then chilled at 0°C overnight. Precipitation occurred in some of those tubes containing antiserum, but not in the tubes with normal serum. That the precipitation was caused by a combination of antigen and antibody and was not a reprecipitation of dissolved immune precipitate by mass action effect was shown by the fact that saline washings of Type I Pneumococcus

---

[1] Liu, S. C., and Wu, H., *Chinese J. Physiol.*, 1938, **13**, 449.

[2] Liu, S. C., and Wu, H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 144.

TABLE I.

Recovery of Antigen from Type I Pneumococcus Immune Precipitate at Different pH's in the Presence of 1% NaCl.
Temperature = 23°C.

pH	2.38	2.68	3.02	3.38	3.88	—	9.72	9.86	10.22	10.65	10.92
Precipitate,* mg N	0.154	0.084	0.042	0	0		0	0	+	+	+
Polysaccharide recovered,† mg	0.20	0.10	0.05	0	0		0	0	+	+	+

*Precipitate formed by the recovered antigen with 1 cc antiserum.

† Read from a control precipitin curve.

0 = No precipitation.

+ = Definite precipitation but the amount was too small to be estimated.

immune precipitate, when mixed with the homologous antiserum, gave no precipitation under the same experimental conditions.

The precipitates were centrifuged at 0°C and washed twice with 1 cc saline also at 0°C. The N contents of the precipitates were determined by micro-Kjeldahl method. A control precipitin experiment was done by adding known amounts of the same lot of Type I Pneumococcus polysaccharide to the same lot of antiserum as that used in preparing the original immune precipitate under the same experimental conditions. The amount of precipitate N was plotted against the amount of polysaccharide. The amount of polysaccharide recovered at different pH's in the recovery experiment was then read from the control precipitin curve. The results are shown in Table I. It will be noted that antigen was recovered only in comparatively more acid solutions, where the recovery of antibody was previously shown[2] to be over 50%. When the pH was higher than 3.38, the amount of antigen recovered was too small to be estimated accurately.

Summary. Antigen was recovered from Type I Pneumococcus immune precipitate from which some antibody had been removed by treatment with acid. This experiment substantiates our previous finding that the recovery of antibody from immune precipitate by the action of acid or alkali is due to a shift in the antigen-antibody equilibrium. The present finding also suggests a possible method for the isolation of pure antigen which may be useful when it cannot be obtained otherwise.

11324

Further Studies on Type-Specific Protein of *Corynebacterium diphtheriae.*

Sam C. Wong and T. T'ung. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

In a previous study[1] it was found that a serologically active, type-specific protein could be prepared by mild alkaline extraction at low temperature from the well-known Park 8 strain. In view of this finding it seems of interest to extend this observation to other types of *C. diphtheriæ* in order to determine (1) whether the method of extraction has a general application and (2) whether the protein is responsible for type-specificity of all types.

---

[1] Wong, Sam C., and T'ung, T., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 824.

Method. The organisms employed for the present investigation were selected at random from the representative types of Sia and Huang's scheme of classification of *C. diphtheriæ*.[2] Seven strains belonging to 5 serological types and including the cultural types of gravis, intermediate and mitis were used. All but one (strain No. 7) were virulent. The growing of the organisms, the method of preparing the protein antigens, and the production of immune sera with the 7 strains were the same as those previously outlined.[1] In addition, immune sera of the following strains were also prepared: 8, 9, 10, 11. In all instances 2 rabbits for each strain, making a total of 22, were used. The precipitin ring test was performed at room temperature by carefully laying various dilutions of the antigen over a constant amount of undiluted immune serum and the results, read 2 hours later. The protein content of each preparation was estimated by determining the total nitrogen with the Micro-Kjeldahl method. The nitrogen value was multiplied by the protein factor 6.25 to give the concentration in milligrams. In the table one mg of protein is expressed as 1:1000 dilution.

In the course of this investigation several difficulties arose. In the first place the diphtheria bacilli are essentially poor antigens. While a type-specific agglutinating serum could be obtained generally in about 3-4 weeks of immunization in rabbits, a precipitin serum either for the proteins or for the polysaccharides may require several months. In 2 instances (strains 2 and 4) antibodies could not be detected by various dilutions of the polysaccharide although precipitins were present when tested with the protein antigens at high concentrations. Satisfactory sera for strains 2, 4 and 5 were obtained only after 6 months of immunization. New animals were used for a few of the strains without any apparent success. In the second place, sera of rabbits immunized with *C. diphtheriæ* invariably acquired anticomplementary properties. This fact renders the complement-fixation test inapplicable for the detection of type-specific antibodies. In the third place, serological activities of the proteins varied with different preparations. At times it was almost impossible to decide whether the protein was inactive or whether the immune serum was unsuitable. In general, however, the potency of an immune sera for the specific protein antigen may be estimated by its precipitin titer for the polysaccharide. The higher the titer for the latter the more suitable is the serum for the demonstration of the type-specific antigen.

---

[2] Sia, R. H. P., and Huang, C. H., *Ibid.*, 1939, **41**, 348.

Chemical Studies. All the protein preparations obtained gave the usual color reactions for proteins (biuret, xanthoproteic, Hopkins-Cole, and Millon). They could be precipitated by neutral salts such as ammonium sulfate and sodium sulfate, by heavy metals such as copper sulfate or lead acetate, and by trichloracetic and tannic acids. The Molisch test for polysaccharide in 1:500 dilution of the protein was negative. Extraction of proteins with ether did not destroy type-specificity but their serological activities were reduced. This indicates that the specific component is not a lipoid-complex.

In our previous report[1] we found that the type-specific protein prepared from Park 8 was heat-labile, being converted into a group specific protein upon heating at 56°C for 30 minutes. Subsequent study on this and other type-specific proteins, however, showed that this observation has no general application and appears to vary with different preparations which in turn depend upon a number of undetermined factors. It seems that the more active the preparation the conversion is more likely to occur although complete destruction of serological activity by heat has yet to be observed. Indeed, all the protein preparations employed in the present study in concentrations of 5-10 mg could withstand heating at 122°C for 20 minutes without alteration of type-specificity nor diminution of serological activity. However, the phenomenon of type to group-specific conversion could be demonstrated with certain preparations particularly from some strains (Park 8 and No. 3). Furthermore all proteins prepared by 1% potassium hydroxide extraction of organisms in boiling water bath are group-specific although the serological activity in general

TABLE I.
Type-specificity of Proteins as Shown by Precipitin Reactions.

Serum	Type	Protein						
		1	2	3	4	5	6	7
1	D41	1:100,000	1:5,000	—	—	—	—	—
2*	D41	1: 5,000	1:1,000	—	—	—	—	—
3	D43	—†	—	1:100,000	1:5,000	—	—	—
4	D43	—	—	1: 10,000	1:1,000	—	—	—
5	D14	—	—	—	—	1:5,000	—	—
6*	6287	—	—	—	—	—	1:10,000	—
7*	X	—	—	—	—	—	—	1:5,000
8	D25	—	—	—	—	—	—	—
9	D30							
10	D40							
11	1219							

*Sera which have been previously absorbed.
†Indicates negative to 1:500 dilution of the protein.

was low. The mechanism underlying these reactions is being studied.

Serological Studies. The results are presented in Table I. It is to be noted that all sera except strains 2 and 4 used in these studies were very potent when tested with the polysaccharide, precipitin titers ranging from 1:200,000 to 1:500,000 being observed. An analysis of the data reveals several points of interest. In the first place proteins from certain strains appear to be more active in serological test than others. For example antigens prepared from strains 1, 3, 6, 7 gave a specific reaction with the homologous type sera in dilutions of 1:50,000 to 1:100,000 while those from strains 2, 4 and 5 reacted in only 1:5000. It is conceivable that the marked difference in serological activities of the various antigens may be due either to denaturation of the antigen during preparation or to unsuitable serum. In the second place none of the proteins reacted with heterologous type-sera produced with strains 8, 9, 10 and 11. In the third place all the protein antigens are strictly type-specific with the exception of those from strains 6 and 7. Even here there was a quantitative difference, the protein reacting with the homologous serum to a much higher titer. In order to show the type-specificity, reciprocal absorption tests were carried out between the undiluted immune sera of strains 2, 6 and 7 and the organisms. The results showed that the common precipitins were promptly removed. The titers of the resulting type-specific sera, however, were significantly lowered when retested with the homologous protein. In case of sera of strains 6 and 7 there was a ten-fold drop in the precipitin titer. This may explain the marked cross reactions observed in the slide agglutination test with these strains.

Comment. The result of the present study shows clearly (1) that the method, consisting of alkaline extraction of bacilli previously defatted, is applicable to all the types studied and (2) that the protein is the cellular component responsible for type-specificity. For the demonstration of the latter point, serum from superimmunized animals in general is necessary. Furthermore, if marked cross reaction occurs in the agglutination test it is also likely to occur with the protein antigens. The difference, however, is quantitative and the common antigenic factor could be easily removed by reciprocal absorptions between immune serum and the heterologous organism. It may be mentioned that the third cellular component of *C. diphtheriæ,* the lipoids, obtained from all the 7 strains are, like the polysaccharides,[3] group-specific. This is in agreement with the observation previously reported on Park 8.[1]

---

[3] Wong, Sam C., and T'ung, T., *Chinese Med. J.*, Suppl. III, 1940, in press.

Conclusion. From the observations made on the chemical and immunological studies of the polysaccharide, lipoid, and protein fractions obtained from representative serological and cultural types it is justifiable to conclude that the alkali-soluble protein is the cellular constituent responsible for type-specificity in *C. diphtheriæ.*

11325

Thyroid Enlargement Following Liver Feeding in Rats.

H. C. Hou.

From the Division of Physiological Sciences, Henry Lester Institute of Medical Research, Shanghai.

That the feeding of liver to animals produces an enlargement of the thyroid gland was reported by Hunt,[1] who found that the thyroid of mice fed on a diet of oatmeal and liver was larger and more vascular than of those on a diet of eggs, crackers and milk. Marine[2] produced a hyperplasia of the thyroid in brook trout by feeding them with liver and heart. Burget[3] found that rats kept under hygienic conditions and fed a high protein diet, consisting of fresh liver and lean beef mixed with a little oatmeal or bread crumbs, developed a hyperplasia of the thyroid. All the above reports of enlargement of the thyroid were interpreted as due to the high protein diet.

Recently, Remington[4] reported that in a low iodine goiter-producing diet, the replacement of a part of the wheat gluten by dried pig liver aggravated the degree of goiter, while purified casein prevented goiter formation, and yeast had no effect. This led Remington to believe that the liver in his goiter-producing diet was responsible for the enlargement of the thyroid. The writer (Hou[5]) in the same year reported that rats fed on a soybean-millet diet with dried liver powder as the only source of animal protein developed a marked hypertrophy of the thyroid gland. The enlargement was over 10 times when the animal was fed on the millet and liver diet for over a year. It becomes of great interest to know which constituent or constituents of the diet is or are responsible for the enlargement of the thyroid. The following series of experiments were accordingly carried out.

---

[1] Hunt, Reid, *J. Am. Med. Assn.*, 1911, **57**, 1032.

[2] Marine, D., *J. Exp. Med.*, 1914, **19**, 70.

[3] Burget, G. E., *Am. J. Physiol.*, 1917, **44**, 492.

[4] Remington, R. E., *J. Nutrition*, 1937, **13**, 223.

[5] Hou, H. C., *Chinese J. Physiol.*, 1936, **10**, 659; 1937, **12**, 488.

Albino rats of the Wistar strain were selected with reference to litter mates and sex when about one month old. Five rats, each from a different litter, were put into one group. The litter mates were put into 3 other groups. The 4 groups were given 4 different diets as follows:

A7	Soybean Flour	450
	Millet	500
	Sodium Chloride	20
	Dried Ox Liver Powder	30

A7a	Soybean Flour	450
	Millet	500
	Sodium Chloride	20
	Dried Beef	30
	Carotene (0.008 mg per rat per day)	
	Ostelin	2 cc

A7b	Soybean Flour	450
	Millet	500
	Sodium Chloride	20
	Dried Ox Liver (extracted with Alcohol)	30
	Carotene (0.008 mg per rat per day)	
	Ostelin	2 cc

A7c	Soybean Flour	450
	Millet	500
	Dried Beef	30
	Sodium Chloride	20
	Residue of Alcohol Extract of Ox Liver (from 30 g of dried liver)	
	Ostelin	2 cc

The rats were kept on these diets for 3 months and then sacrificed. The thyroid glands were weighed with a micro-analytical balance immediately upon their removal from the body. The weights of thyroid glands of the various groups are shown in Table I.

It will be noted that rats fed on diet A7 had the largest thyroid, next rats on A7c, then those on A7a. Rats fed on A7b had the smallest thyroid which is within the normal range of thyroid weight. From these results it appears that the dried ox liver was the constituent in the diet which caused the marked enlargement of the thyroid since the substitution of liver by dried beef resulted in only a slight

TABLE I.
Influence of Liver and Liver Extract on Thyroid Gland.

	A7		A7a		A7b		A7c	
	(Dried ox liver)		(Dried beef)		(Extracted liver)		(Alcoholic extract of liver	
	Body wt g	Thyroid mg	Body wt g	Thyroid mg	Body wt g	Thyroid mg	Body wt g	Thyroid mg
	127	58.7	96	14.8	57	9.2	110	31.8
	98	53.8	88	19.0	150	16.0	120	38.3
	138	55.5	93	24.3	164	17.0	130	35.4
	108	33.8	156	42.9	158	13.5	140	50.0
	90	29.7	113	23.5	147	18.1	111	28.3
Avg	112	46.3	109	24.9	135	14.8	122	36.7
Thyroid mg/100 g Body wt.	41.3		22.8		10.9		30.1	

enlargement of the thyroid. Furthermore, when the liver had been extracted with alcohol (diet A7b), its feeding did not induce any enlargement of the thyroid. On the other hand, the inclusion of the residue from the alcoholic extract of liver in the diet containing dried beef (A7c) caused the thyroid to increase in size to a degree approximating the enlargement due to the dried liver diet (A7).

It appears, therefore, that the factor which causes an enlargement of the thyroid in rats lies in the alcohol-soluble portion of the dried liver. The enlargement as shown by histological examination was general in character with the organ a little more vascular and cellular than normal. Further experiments are being carried out to determine the actual substance present in the alcohol-soluble portion of liver which causes the enlargement of the thyroid.

11326 P

Cultivation of Virus of Encephalitis (St. Louis Type) on Agar Tissue Medium.

K. H. Pang. (Introduced by S. H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping.

Although the virus of encephalitis of St. Louis type has been successfully cultivated by Maitland technic and on the chorio-allantoic membrane of developing chick[1-4] the titer obtained in these cultures has been invariably low. None of the cultures showed a titer as high as 10^{-6} even after repeated passages. Recently Kawakita[5] has reported successful cultivation of Japanese encephalitis virus in a special medium containing chick allantoic fluid. It has occurred to us to attempt to cultivate the virus of St. Louis type on the agar-tissue medium which has been found to be satisfactory for the growth of various types of rickettsiae.[6, 7] The results of such a study together

---

[1] Syverton, J. T., and Berry, G. P., *Science*, 1935, **82**, 596.

[2] Harrison, R. W., and Moore, E., *Am. J. Path.*, 1937, **13**, 361.

[3] Schultz, E. W., Williams, G. F., and Hetherington, A., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 799.

[4] Smith, M. G., Proc. Soc. Exp. Biol. and Med., 1939, **40**, 191.

[5] Kawakita, Y., *Jap. J. Exp. Med.*, 1939, **17**, 211.

[6] Zinsser, H., FitzPatrick, F. K., and Wei, H., *J. Exp. Med.*, 1939, **69**, 179.

[7] Zia, S. H., Liu, P. Y., and Pang, K. H., *Chinese Med. J.*, 1938, **54**, 547.

with a preliminary study on the immunizing value of vaccine prepared from these cultures are hereby communicated.

The virus, Webster No. 3, which has full virulence for mice, was propagated in the following fashion: Infected mouse brain, diluted with serum-Tyrode solution mixture (1:5) to 10% by weight was filtered through Seitz E. K. pad after brief centrifugalization. A small amount of the filtrate was inoculated on to a small piece of young mouse embryonic tissue in a sterile Petri dish. These were minced together, and after being allowed to stand for 15 to 20 minutes at room temperature, the finely cut tissue bits were carefully laid on the surface of agar slants employed for the cultivation of rickettsiae. These cultures were incubated at 37°C, and transferred every 5-8 days. By this method, the virus has been carried for over 40 generations in a period for more than 10 months. The activity of the virus for Swiss mice by intracerebral injections of different generations was found to be as follows: 10^{-6} for the sixth, 10^{-6} for the sixteenth, 10^{-7} for the twenty-first, and 10^{-6} for the thirty-fourth generations of cultures. It was also found that as little as 10^{-6} or 10^{-7} dilution of the filtrate of infected mouse brain or only a few M.L.D. for mice, could successfully initiate cultures on agar tissue medium. These observations showed conclusively that there has been satisfactory multiplication of the virus on embryonic tissue lying on the surface of agar containing serum and Tyrode solution, and that the titer in each generation might reach that of infected mouse brain. Furthermore, relatively small amount of virus may initiate these cultures which suggests that the cultural method might be applicable in the isolation of virus.

Vaccine was prepared by grinding the tissue bits collected from several tubes and preserved with 0.1% formalin. Each cc of the vaccine contained materials which had 10,000 M.L.D. for mice before inactivation. Immunization experiments have been attempted with 10 mice inoculated intraperitoneally at 3 days' interval with 2.5 cc and another 10 mice with 1.5 cc of the vaccine. One week after the last injection, the mice received intracerebral injections of 1,000 M.L.D. for mice. So far, none of the animals that received culture vaccine survived the infecting dose. However, the possibility of employing this cultural method for the preparation of successful vaccine remains to be further exploited.

Comment. In contrast to the results of previous workers, it has been possible, by means of the agar tissue medium, to cultivate the virus of encephalitis of St. Louis type, for over 40 generations with no decrease in its virulence for mice. It has also been possible to obtain higher titer of multiplication than was possible by previous methods. Attempts have been made to utilize the cultured virus for the preparation of immunizing vaccine but so far only equivocal results have been obtained. With the recent report of Kurotchkin[8] on the successful cultivation of vaccinia virus on the same medium, further trials with some other viruses seem to be indicated.

11327 P

Ultraviolet Absorption Spectrum of Cytochrome C.

GEORGE I. LAVIN, CHARLES L. HOAGLAND AND S. M. WARD.
(Introduced by O. T. Avery.)
From the Laboratories and Hospital of the Rockefeller Institute for Medical Research, New York.

Dixon, Hill and Keilin[1] published the ultraviolet absorption curve of cytochrome c. A sharp maximum was found at about 4150 Å. Other than a plateau in the region 2600-2900 Å no other structure was detected. Roche and Benevent[2] also found a similar curve.

The cytochrome c used was prepared from fresh beef heart by the method of Keilin and Hartree.[3] Tetrapyrrolic iron was determined by the dipyridyl technic of Hill,[4] following the treatment of cytochrome c with alkali and hydrogen peroxide. Several lots were assayed for the hemin iron content with an average value of 0.39%. The theoretical value is around 0.4%.[5]

Since it is known that the absorption curves obtained with the line source and photometer may fail to show all the details of the bands,[6] photographs were taken with the continuous light of the hydrogen discharge tube and a small Hilger quartz spectrograph. The spectrum obtained is drawn in Fig. 1a. The band at 4150 Å is seen, also other bands not clearly indicated in the curve. The band in the region of 3500Å is rather diffuse as are the other bands in the protein region. Not shown in the illustration are bands in the region 2550-2600 Å. These are of such intensity that it is difficult to obtain their exact wave length.[5]

---

[8] Kurotchkin, T. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 407.

[1] Dixon, M., Hill, R., and Keilin, D., *Proc. Roy. Soc.* B, 1931, **109**, 29.

[2] Roche, J., and Benevent, M. T., *Bull. Soc. Chim. Biol.*, 1935, **17**, 1473.

[3] Keilin, D., and Hartree, E. F., *Proc. Roy. Soc.* B, 1937, **122**, 298.

[4] Hill, R., *Proc. Roy. Soc.* B, 1930, **107**, 205.

[5] Theorell, H., *Science*, 1939, **90**, 67.

[6] Lavin, G. I., and Northrop, J. H., *J. Am. Chem. Soc.*, 1935, **57**, 874.

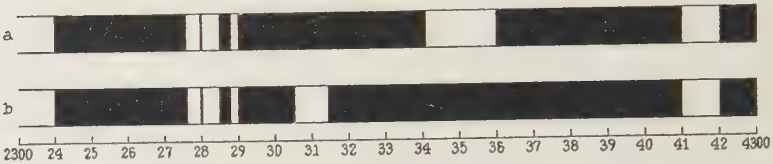


Fig. 1

When cytochrome c is treated with sodium formaldehyde sulfoxylate ($CH_2OHOSONa$) the band at 3500 Å disappears and a new band appears at about 3100 Å, as depicted in Fig. 1b. At the same time the well characterized band of reduced cytochrome c becomes apparent in the visible at 5500 Å. Experiments are now in progress to determine the possible reversibility of this process and the complete significance of the above data is being further investigated.

11328

A Technique for the Perfusion of the Foetal Placental Circulation.*

R. F. DAWSON AND J. M. ROBSON. (Introduced by A. J. Clark.)

From the Department of Pharmacology, University of Edinburgh.

Investigation of the various factors which control the placental circulation has necessarily been limited owing to the experimental difficulties involved. It is suggested that the following technic may offer a line of approach to some of the problems involved.

Methods. The experimental animals used were cats or bitches which were in an advanced stage of pregnancy. Rabbits were found to be unsuitable owing to the friability of the placental vessels. Anesthesia was induced with open ether, and maintained with chloralose given intravenously in a dose of 100 mg/kilo body weight. Both vagi were cut, and the carotid blood pressure recorded by means of a mercury manometer. The abdomen was opened with as short an incision as was compatible with adequate access, and a part of the uterus containing one foetus brought up into the wound. A small cork platform approximately 5 cm square was approximated to the section of uterus exposed, and the uterine peritoneum loosely stitched to the two nearest corners of the platform with silk ligatures. An incision about 2 cm long was then made through a part of the uterine wall which was free of placental attachments, the membranes ruptured, and the hind-quarters of the foetus delivered until the umbilical cord came into view. If the foetus was lying in a favorable position, it was possible to expose the umbilicus with the umbilical vessels without delivering any part of the foetus. The umbilical cord was then ligatured in 2 places as close to the umbilicus as possible, divided, and the placental end delivered through the wound in the uterine wall. Thereafter the foetus was replaced within the uterus and the uterine wall stitched up again, leaving only the free end of the umbilical cord protruding. With practice it was possible to perform this operation without any appreciable loss of amniotic fluid, so that the intrauterine pressure was not materially altered.

---

* The expenses of this investigation have been defrayed by a grant from the Medical Research Council.

The vessels of the cord were cleaned, a cannula was inserted into the umbilical artery and perfusion of the placental circulation immediately started by means of a small Dixon pump. The perfusion apparatus was the same as was used by Robson and Schild.[1] Another cannula was rapidly inserted into the umbilical vein to collect the perfusion fluid and thus prevent distention or clotting within the placental circulation.

The inflow perfusion pressure was recorded on the kymograph by a mercury manometer. The blood passing from the pump to the umbilical artery was kept at body temperature by passing the rubber tubing, which connected the two, through the heating apparatus of the operating table. The venous return from the umbilical artery was allowed to flow by gravity through the cannula and rubber tubing to the reservoir of the pump. The dead space of the perfusion apparatus (about 20 cc) was filled either with saline or with blood taken from the maternal circulation. Coagulation was prevented by means of repeated additions of heparin to the contents of the reservoir. Approximately 4 mg of heparin dissolved in 1 cc Ringer-Locke solution were required every half to one hour. The cannulae were maintained in position by skewering them to the cork platform, and this insured that there was no kinking of or traction upon the umbilical vessels.

The contractions of the part of the uterus containing the foetus were simultaneously recorded by means of a Cushny myograph. By the use of a small celluloid window and pads of cotton wool, the abdominal contents were maintained at a normal body temperature.

The minute volume of the circulation in the full-term foetus has been estimated to be of the order of 0.1 cc/g weight of the foetus in goats (Barcroft, *et al.*[2]). The weight of the foetus of the cat in the later stages of pregnancy is about 50 g. In order to insure an adequate blood flow through the placenta the Dixon pump was adjusted to deliver about 8 cc/min., and at this rate the perfusion pressure was usually approximately 80 mm Hg.

---

[1] Robson, J. M., and Schild, H. O., *J. Physiol.*, 1938, **92**, 9.

At the end of each experiment the adequacy of the perfusion was checked by injection of methylene blue into the venous cannula, and subsequent examination of the placenta. In all cases it was found that the perfusion had been satisfactory.

Results. When 1 μg of acetyl choline was injected intravenously into the maternal circulation it cause a small and transitory fall in the maternal blood pressure, but had no effect upon either the uterine contractions or the perfusion pressure. Ten μg caused a greater fall in the maternal blood pressure, but again did not affect either uteri contractions or perfusion pressure. When 1 μg of acetyl choline was injected into the arterial cannula of the perfusion system, a slight and transitory rise occurred in the perfusion pressure, and this effect was greater and more prolonged when the amount of acetyl choline injected was increased to 100 μg. In neither case was there any demonstrable effect upon either the uterine contractions or the maternal blood pressure.

Adrenalin was injected into the perfusion system in doses of 1 to 100 μg. In one experiment a sustained rise in perfusion pressure followed the injection of 10 μg of adrenalin, but in none of the other experiments did the administration of the drug produce any effect on the perfusion pressure, the maternal blood pressure, or the uterine contractions.

0.1-1.0 unit pituitary (posterior lobe) extract injected into the maternal circulation caused a prolonged rise in the maternal blood pressure, and an increase in both the rate and the amplitude of the uterine contractions, with a consequent increase in the rate and amplitude of the variations in the perfusion pressure. This is illustrated in Fig. 1. When 1 unit was injected into the perfusion system it caused a sustained rise in perfusion pressure, with, in one case, an increased rate of uterine contractions. In no case was any effect on the maternal blood pressure noted.

A constant observation in these experiments was that cyclical variations in perfusion pressure occurred synchronously with the uterine contractions. This is in contradistinction to the findings during artificial perfusion of the maternal-uterine circulation, where uterine tone and motility have no appreciable effect upon the perfusion pressure (Robson and Schild[1]).

---

[2] Barcroft, J., Kennedy, J. A., and Mason, M. F., *J. Physiol.*, 1939, **95**, 269.

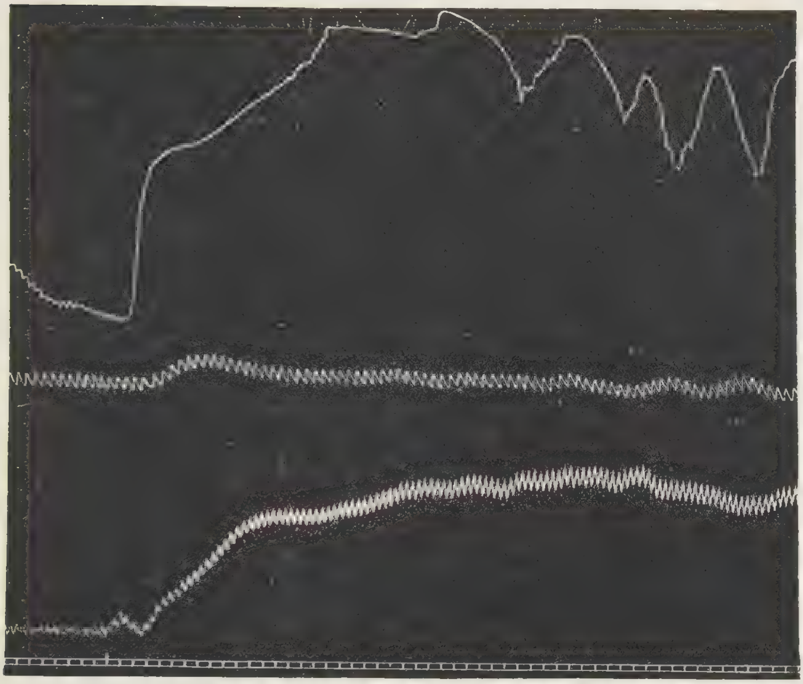


Fig. 1.

Cat Sb 38. Upper tracing: Uterine contractions. Middle tracing: Perfusion pressure. Lower tracing: Maternal blood pressure. Signal: 1 unit pituitary (posterior lobe) extract injected intravenously into maternal circulation. Time intervals: 1 min.

In addition to these variations in perfusion pressure it was also observed that the return of blood from the placenta into the reservoir of the perfusion pump was not a continuous flow, but was at a maximum during the period of uterine relaxation and during the early part of a contraction, and then sharply decreased as the contraction developed. These results suggest that uterine tone and motility may play a part in aiding the return of blood from the placenta during the later stages of pregnancy. The results also offer evidence that the blood flow through the foetal placenta is markedly decreased by the contractions of the uterus.

Clark[3] found that during uterine contractions there occurred a transient rise followed by a fall in the foetal blood pressure, and ascribed the fall to a reduced venous return from the placenta to the heart. This is in agreement with our own results on the return of blood from the placenta during uterine contractions.

---

[3] Clark, G. A., *J. Physiol.*, 1932, **74**, 391.

11329

Effect of Metrazol on Cerebral Vessels.

HENRY S. FORBES AND GLADYS I. NASON.

From the Department of Neuropathology, Harvard Medical School, Boston.

At the beginning of a convulsive seizure in animals, caused by metrazol (pentamethylenetetrazol), pallor of the brain surface has been reported. Liebert and Weil attribute this to "vasospasm".[1] Watterson and Macdonald, on the other hand, find "no certain evidence that the convulsion is either caused or accompanied by cerebral vasoconstriction," but they do find that drugs causing vasodilation tend to inhibit metrazol seizures.[2]

To get more accurate data on the vascular response to metrazol 15 cats and one monkey have been studied as follows: The diameters of pial arterioles and venules over the parietal cerebral cortex were measured by microscope through a cranial window.[3] Metrazol

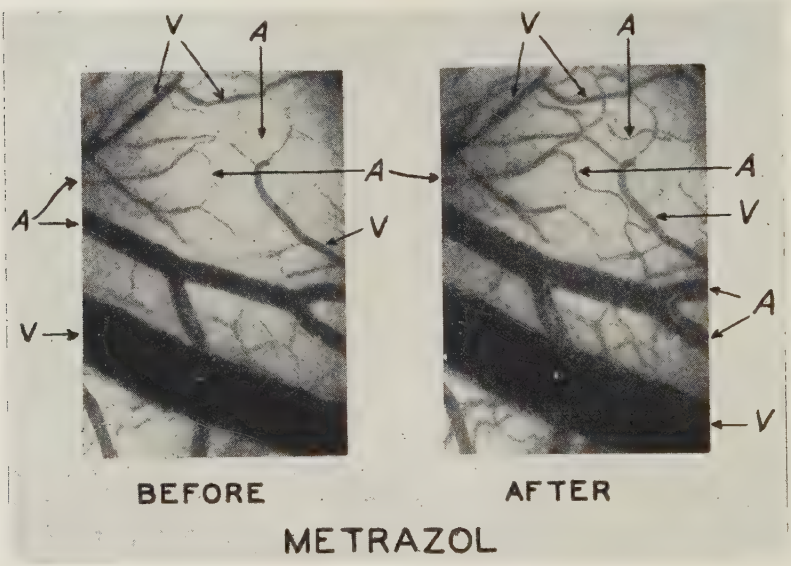


FIG. 1.

Photomicrographs of pial vessels before and after intravenous injection of 4% metrazol (40 mg per kilo). The arteries (A), veins (V) and their branches are all dilated in the second photo taken 65 seconds after the injection. Taken on 35 mm film—ocular ×10, objective ×3.

---

[1] Liebert, E., and Weil, A., *Arch. Neurol. and Psychiat.*, 1939, **42**, 690.

[2] Watterson, D. J., and Macdonald, R., *J. Ment. Sci.*, 1939, **85**, 392.

[3] Forbes, H. S., *Arch. Neurol. and Psychiat.*, 1928, **19**, 751.

(8 to 80 mg per kilo, in 4% solution) was injected into the saphenous vein or carotid artery (46 trials), or it was applied locally to the surface of the brain (5 trials). Diallyl barbituric acid was used for anesthesia except in two instances, when intraperitoneal magnesium sulphate was used.* Cerebrospinal fluid pressure from the cisterna magna was recorded by photokymograph.[4]

The findings were these: The pial arteries (and veins) dilated (Fig. 1). The dilation usually started a few seconds before the first twitches were seen. Occasionally the seizure appeared first (Fig. 2). After a 5-second intravenous injection of metrazol the latent period before the first sign of dilation averaged 10 seconds (26 trials). The latent period before the seizure averaged 15 seconds.

Sometimes, however, the vasodilation had passed and the vessels were contracting when the convulsion started (Fig. 3) and some-

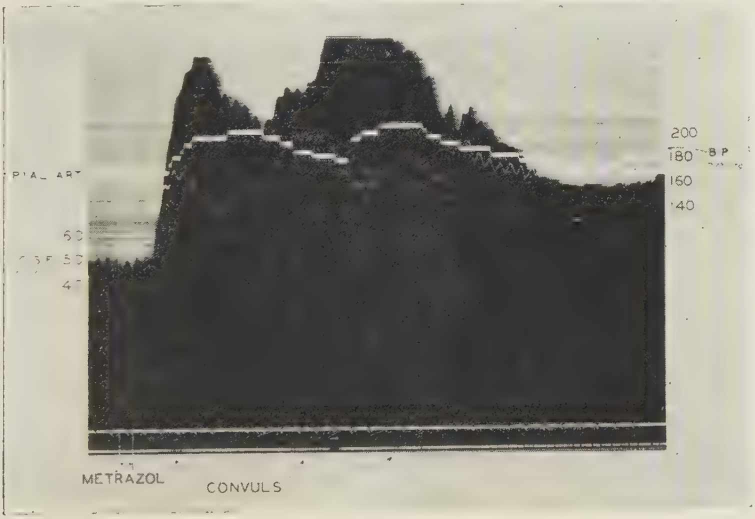


Fig. 2.

Dilation of pial artery. *Early convulsion.* Magnesium sulphate anesthesia. Artificial respiration. Metrazol—17 mg per kilo (1 cc 4% solution) was injected into a leg vein. Cerebrospinal fluid pressure (C.S.F.) is recorded by the top edge of the black area, which is the photographic tracing of the meniscus of fluid in a 1 mm bore manometer connected with the cisterna. Blood pressure was taken from the right common carotid. First convulsive twitches occurred one or 2 seconds before the pial artery started to dilate. No consistent relationship is apparent between C.S.F. pressure, blood pressure and arterial calibre.

---

* The magnesium sulphate animals did not react to metrazol differently from the others except by showing, often, a rise in blood pressure.

[4] Forbes, H. S., Nason, G. I., and Wortman, R. C., *Arch. Neurol. and Psychiat.*, 1937, **37**, 334.

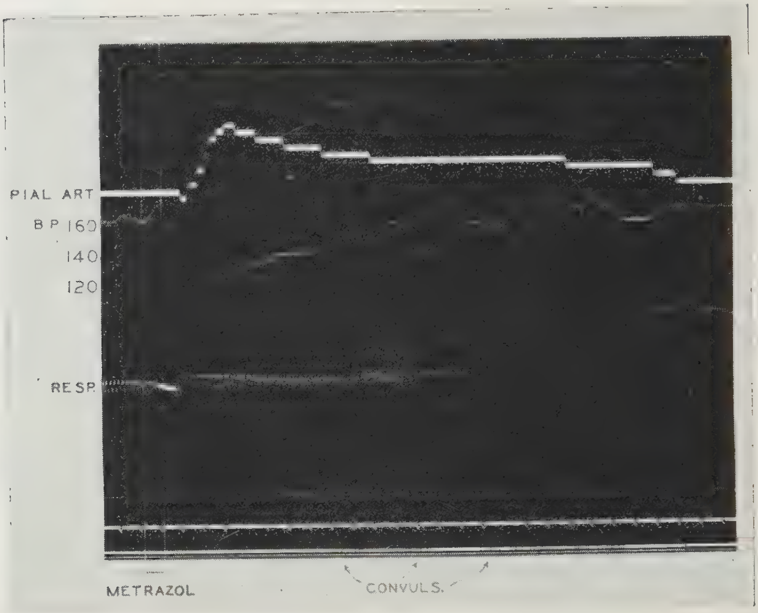


FIG. 3.

Dilation of pial artery. *Late convulsion.* Dial anesthesia. Metrazol—40 mg per kilo (2 cc 4% solution) injected into a leg vein caused sudden fall in blood pressure. Diameter of this pial artery was 254 microns. Blood pressure was taken from the right common carotid. Time marker records 5-second intervals. Breaks in signal line (at bottom) indicate the presence of clonic muscular contractions. The latent period before this seizure was 3 times longer than usual.

times the dilation occurred without any convulsion (Fig. 4). In other words, no constant relation was found between the seizure and the vascular change. Vasoconstriction was not observed.

The systemic arterial pressure, after a period of fluctuation, usually fell. Sometimes it remained almost unchanged. Respirations often stopped for 5 or 10 seconds and then became very rapid. In some experiments artificial respiration (with bilaterally opened thorax) was carried out. Neither the incidence of convulsions nor of vasodilations was affected by changes in respiration or in blood pressure.

Leibel and Hall found a decrease in cerebral blood flow (in rabbits) immediately following the injection of metrazol.[5] They also noted a fall in blood pressure. Undoubtedly this fall was the cause of the decreased flow, which took place in spite of the local vasodilation. In those experiments of ours in which the blood pressure

---

[5] Leibel, B. S., and Hall, G. E., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 894.

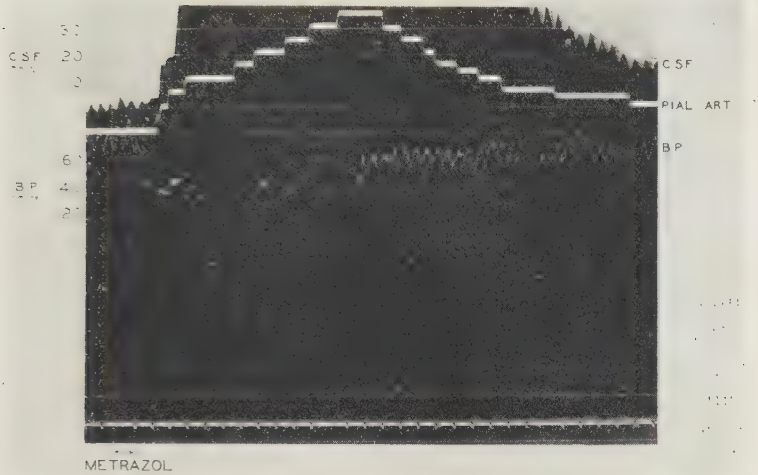


Fig. 4.

Dilation of pial artery. *No convulsion.* Dial anesthesia. Artificial respiration, open thorax. Metrazol—50 mg per kilo (3 cc 4% solution) injected into a leg vein caused a pronounced fall in blood pressure. The pial artery (104 microns in diameter) dilated promptly but no convulsion occurred. The rise in cerebrospinal fluid pressure—recorded by top of black area—happened in this instance to synchronize with the period of vasodilation. This relationship was not constant, however.

remained constant, or rose, it is fair to assume that the flow increased.

The rise in cerebrospinal fluid pressure, after each metrazol injection, showed no constant relation to any of the other variables which were measured (Fig. 2).

Summary. In animals metrazol caused cerebral vasodilation. This was unrelated to changes in blood pressure or in respiration, and showed no constant relation to convulsive seizures. No cerebral vasoconstriction was observed.

11330 P

Configuration of Glutamic Acid Isolated from Subacute Lymphatic Leukemic Tissue Proteins.

L. Earle Arnow, Jeanette C. Opsahl and C. J. Watson.

From the Laboratories of Physiological Chemistry and Internal Medicine, University of Minnesota, Minneapolis.

The exact relationship of leukemia to neoplastic disease has not been determined. The following types of human neoplasm, when examined by the procedure used by Kögl and Erxleben,[1] have been reported to yield partly racemized glutamic acid:[2] carcinoma of the ovary, carcinoma of the breast, adenocarcinoma of the colon, and sarcoma of the thigh. Normal human tissues (ovaries, colon) have been reported to yield only 1(+)glutamic acid.[2]

We have isolated glutamic acid from the liver and spleen of a patient who died of subacute lymphatic leukemia. Microscopic examination revealed that at least 20% of the liver, and 30% of the spleen, were composed of leukemic areas. Major portions of these organs removed at autopsy were placed in alcohol. Following transport to the laboratory, the tissues were finely minced, after which they were covered with 0.1 *N* hydrochloric acid and heated several hours on the steam bath until a majority of the material had gone into solution. Sufficient trichloracetic acid then was added to make a concentration of 10%. The precipitated proteins were filtered off, washed and extracted several times with hot alcohol in order to remove trichloracetic acid. The proteins were dried at 110° and lipids partly were extracted with boiling ether. Hydrolysis and isolation of glutamic acid were then carried out as described by Kögl, Erxleben, and Akkerman.[3] The results obtained are recorded in Table I. No racemic acid was isolated from the leukemic tissues.

TABLE I.

	Melting point uncorrected	$[\alpha]_D$* (in 9% HCl)	% of racemic glutamic acid
Leukemic liver	203-204°	+31.3°	0
,, spleen	203-204°	+31.0°	0
Sarcoma (dog kidney)	201-202°	+27.8°	12

*Literature: +31.7°, calculated for free 1(+) glutamic acid.

---

[1] Kögl, F., and Erxleben, H., *Z. physiol. Chem.*, 1939, **258**, 57.

[2] Kögl, F., *Z. Krebsforsch.*, 1939, **49**, 291; Arnow, L. E., and Opsahl, Jeanette C., *Science*, 1939, **90**, 257; White, J., and White, F. R., *J. Biol. Chem.*, 1939, **130**, 435.

[3] Kögl, F., Erxleben, H., and Akkerman, A. M., *Z. physiol. Chem.*, 1939, **261**, 141.

As a control, glutamic acid was isolated from protein obtained from a kidney neoplasm (dog). We are indebted to Dr. E. T. Bell, Head of the Department of Pathology, University of Minnesota Medical School, who examined sections of this tumor and found it to be an undifferentiated sarcoma.

11331

Configuration of Glutamic Acid Isolated from Proteins of Pig and Chick Embryo Tissues.

WILLIAM G. CLARK, L. EARLE ARNOW AND JEANETTE C. OPSAHL.
(Introduced by F. H. Scott.)

Laboratories of Zoology and Physiological Chemistry, University of Minnesota, Minneapolis.

Since Kögl and Erxleben[1] announced the isolation of partly racemized glutamic acid from malignant tissue protein, numerous confirmatory and non-confirmatory articles have appeared. The only laboratories, other than Kögl's, that have investigated carcinoma protein by the Kögl-Erxleben procedure have reported the isolation of partly racemized glutamic acid.[2] Dittmar[3] obtained only 1(+)glutamic acid from Rous sarcoma and from Jensen sarcoma free of necrosis, but later[4] found racemic glutamic acid in mouse sarcomas and carcinomas containing some necrotic material. Johnson[5] reported the isolation of glutamic acid containing small percentages of racemate from acid hydrolysates of Jensen sarcoma, but claimed that similar percentages were obtained also from normal mouse liver protein hydrolysates.

Kögl, Erxleben, and Akkerman[6] found only 1(+) glutamic acid in the hydrolysate of protein obtained from two- to three-months-old calf embryos. The embryonic tissues of other animals have not been investigated from this point of view. Since the metabolism of malignant tissue is similar in many respects to that of embryonic tissue, obviously it is important to investigate the configuration of the glutamic acid obtained from the latter.

---

[1] Kögl, F., and Erxleben, H., *Z. physiol. Chem.*, 1939, **258**, 57.

[2] Arnow, L. E., and Opsahl, J. C., *Science*, 1939, **90**, 257; White, J., and White, F. R., *J. Biol. Chem.*, 1939, **130**, 435; Dittmar, C., *Z. Krebsforsch.*, 1939, **49**, 441.

[3] Dittmar, C., *Z. Krebsforsch.*, 1939, **49**, 397.

[4] Dittmar, C., *Z. Krebsforsch.*, 1939, **49**, 441.

[5] Johnson, J. M., *J. Biol. Chem.*, 1940, **132**, 781.

[6] Kögl, F., Erxleben, H., and Akkerman, A. M., *Z. physiol. Chem.*, 1939, **261**, 141.

We have isolated glutamic acid from the proteins of pig and chick embryo tissues. The isolations proved unexpectedly difficult, and glutamic acid hydrochloride crystallized only after standing in saturated hydrochloric acid solution for several weeks. Nine percent hydrochloric acid was used as a solvent in the determinations of optical rotation. The specific optical rotations reported in Table I have been calculated for free glutamic acid (literature: + 31.7° for 1(+)glutamic acid). The melting point (more properly, the decomposition point) of glutamic acid hydrochloride varies somewhat, depending on the method used for its determination. By the method employed by us, known pure 1(+) glutamic acid hydrochloride melts at 203-205°.

TABLE I.

	Protein, g	Glutamic acid HCl (Final yield) mg	Melting point	$[\alpha]_D$
Pig	9.2	37.4	203-204°	+31.8°
Chick	15.7	234.1	203-204°	+31.7°

Experimental. Pig Embryos. Pig embryos varying in length from 750 to 1000 mm, and in weight, from 20 to 50 g each, were used. The bones were just beginning to calcify. Two hundred g of fresh total embryo tissue were macerated to a brei in a hand coffee mill. The brei was placed in a heavy cloth bag, and immersed several times in about 500 cc of boiling water to which a few drops of concentrated hydrochloric acid had been added. After centrifugation, the precipitated protein was washed with aqueous alcohol solution (1:4) until the washings were free of chloride. The washed protein was dried at 110°, pulverized in a mortar, and washed 3 times with boiling ether. Yield, 9.2 g of protein. Hydrolysis and isolation of glutamic acid hydrochloride were carried out as described by Kögl, Erxleben, and Akkerman.[6]

Chick Embryos. The entire bodies of 704 seven-day-old chick embryos were removed from eggs incubated by a standard method, blotted carefully with filter paper to remove excess moisture, and weighed (397 g). After maceration, the tissue was covered with 0.1 *N* hydrochloric acid, and heated on a water bath until a majority of the material had gone into solution (6-8 hours). Sufficient trichloracetic acid was now added to make a concentration of 10%, and the precipitated protein was allowed to settle. After filtration with suction, the protein was washed repeatedly with alcohol and ether (to remove trichloracetic acid and the majority of adsorbed lipids). The protein thus isolated weighed 15.7 g following drying at 110°. Hydrolysis and isolation of glutamic acid hydrochloride again were carried out by the procedures used by Kögl, Erxleben, and Akkerman.[6]

Summary. Proteins obtained from the embryonic tissues of chicks and pigs have been hydrolyzed, and the glutamic acid hydrochloride obtained from the hydrolysates examined in the polarimeter. The glutamic acid hydrochloride isolated was entirely the 1(+) variety.

11332 P

Effect of Trypsin on Development of *Rana pipiens.*

ALFRED F. BLISS. (Introduced by L. G. Barth.)
From the Department of Zoology, Columbia University.

Proteases secreted by the embryos of numerous species of fish and amphibia are believed to be important factors in bringing about hatching by digestion of the egg membranes. It has been asserted that in teleosts this protease is much like trypsin and so powerful that it attacks the embryo itself.[1] This statement conflicts with the dictum that living organisms are resistant to digestive enzymes. A striking confirmation of the latter view is Northrup's demonstration of the immunity of a wide variety of organisms to concentrated trypsin.[2]

These conflicting statements suggested the desirability of investigating the action of trypsin on the embryologically useful amphibian *Rana pipiens.* Since this anuran appears to possess a hatching enzyme the effect of trypsin on both membranes and embryonic viability was studied. The eggs were stripped of excess jelly on paper toweling and immersed in a 10% solution of dialyzed trypsin made up in 10% Ringer solution. Each experiment involved 25-50 eggs. Table I gives some typical results.

These results are in agreement with Northrup's work in their clear distinction between the susceptibility of the membrane proteins and the resistance of the living organism to trypsin. The possibility, suggested by work on teleosts, of a general embryonic sensitivity to

---

[1] Needham, J., *Chemical Embryology,* 1931, **3**, 1597.

[2] Northrup, J., *J. Gen. Physiology,* 1926, **9**, 497.

TABLE I.

Material	pH	Time, 20°C	Result
1. Eggs in early cleavage stages	*ca.* 6	6 hr	Membranes fragile
2. ” ” ” ” ”	” 8	3-6 ”	Hatched
3. ” ” ” ” ”	”	1½ ”	Membranes fragile
4. Membranes removed from 3	”	6 ”	Normal development
5. 4, plus tr. digestion residue	”	12 ”	Development stopped

digestive enzymes is not substantiated. While this may still be the case in teleosts, it seems more likely that the injurious action of the egg contents on the embryos was due to digestion products.

The parallelism between the effects of trypsin and normal hatching in *Rana pipiens* is in agreement with the theory that normal hatching is due to a digestive enzyme. It should be pointed out in this connection that the power of trypsin to mimic normal hatching makes it useful in large scale chemical work with early stages of anurans where the presence of the difficultly removable membranes may prove a hindrance to analysis or penetration.

11333 P

Effect of Extravasated Antibody upon Antigenicity of Extracts of Virus-Induced Rabbit Papillomas.

John G. Kidd.

From the Laboratories of the Rockefeller Institute for Medical Research, New York, N. Y.

Saline suspensions of virus-induced rabbit papillomas may stimulate the production of specific antiviral antibody when injected intraperitoneally into normal rabbits, as Shope found, even though they contain no pathogenic virus demonstrable by the ordinary test.[1] In experiments of the same sort we found that saline extracts containing infectious papilloma virus in quantity elicited the antibody in much higher titer than extracts in which little or none was present.[2] Other studies already reported from this laboratory have shown that the antibody often extravasates into the large, disorderly papillomas of cottontail rabbits in such quantity as to "mask" the causative virus,[3] and that the antibody can be identified as such in extracts of the growths.[4] With these findings in mind, experiments were undertaken to determine whether the antibody, which accumulates in the papillomas in various amounts depending upon the titer of it in the blood and upon the local vascular conditions determining its extravasation, may not influence the antigenicity of extracts of the growths.

---

[1] Shope, R. E.; *J. Exp. Med.*, 1937, **65**, 219.

[2] Kidd, J. G., Proc. Soc. Exp. Biol. and Med., 1938, **37**, 657; *J. Exp. Med.*, 1938, **68**, 703, 725, 737.

[3] Kidd, J. G., *J. Exp. Med.*, 1939, **70**, 583.

[4] Friedewald, W. F., Proc. Soc. Exp. Biol. and Med., 1939, **42**, 330.

The antigenicity of the papilloma virus, as determined by its capacity to elicit antibody upon intraperitoneal injection into normal rabbits, was found to be markedly reduced when antibody was mixed with it *in vitro* in amounts sufficient to neutralize it. When an excess of antibody was added to a filtrate containing highly infectious virus, the mixture elicited no antibody upon repeated intraperitoneal injections into normal rabbits, although the control mixture containing saline and the same amount of virus (approximately 20,000 infectious doses for each animal) proved highly antigenic.

To procure virus-induced papillomas that were certain to contain the extravasated antibody in quantity, a number of cottontail and domestic rabbits carrying vigorous confluent growths of 2 to 4 weeks' duration were injected intraperitoneally with large quantities of active virus. This greatly raised the titer of serum-antibody but resulted in no new lesions, not only because the rabbits were already partially immune to the virus but because the virus acts only upon epidermis. After the serum-titer had remained for 10 days or more at the high level to which it had been brought the rabbits were killed for material. Although nourished by blood containing much antibody the growths had enlarged steadily—no unexpected finding since circulating antibody is known to be ineffective against virus associated with living papilloma cells.[5] The growths were washed with soap and water and rinsed well to reduce the number of contaminating bacteria, and then cut away with sterile instruments, diced, and saved in 50% glycerol-Locke's solution in the refrigerator. Other rabbits not hyperimmunized but carrying growths of the same duration and derivation, and hence having various but comparatively small amounts of antibody in their blood and presumably little extravasated antibody in the growths, were likewise killed and their papillomas saved. After the growths had been kept in cold glycerol-Locke's for periods up to 2 weeks, 1:10 or 1:20 saline suspensions were made of them. These were centrifugalized lightly and the supernatant liquids, all heavily opalescent but free from gross tissue-debris, were injected intraperitoneally into normal rabbits, the injections being repeated 8 or 9 days later. After a further interval of 8 to 10 days the rabbits were bled from an ear vein and tested for resistance to the papilloma virus, with tests also of the serum for antibody by means of standardized neutralization and complement-fixation tests.[6]

---

[5] Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 63, 79; Kidd, J. G., *J. Exp. Med.*, 1938, **67**, 551.

Materials derived from 10 wild and 11 domestic rabbits were used in 7 comprehensive experiments. The results were consistent and can be summarized together. Extracts of the 7 wild-rabbit papillomas that contained much infectious virus (and by inference little or no free antibody) invariably proved highly antigenic upon intraperitoneal injection into normal rabbits, eliciting antibody in amounts roughly proportional to the quantity of virus injected. Extracts of 3 wild and 6 domestic-rabbit papillomas, which contained in contrast little or no infectious virus demonstrable on test, elicited antibody in small or moderate amounts upon injection into normal rabbits. Extracts of the growths coming from 5 domestic rabbits having notably high serum-antibody titers failed completely to elicit antibody after repeated injections of large amounts (10 cc of 1:10 or 1:20 saline suspensions). The non-antigenic extracts invariably contained considerable amounts of extravasated antibody, as was proved by their capacity to neutralize added virus *in vitro*. The fact was noted incidentally that passively transferred antibody is often responsible for the resistance to the virus that becomes manifest after extracts of papillomas containing much extravasated antibody are injected intraperitoneally into normal rabbits.

The findings would appear to warrant the conclusion that antibody can reduce or abolish the antigenicity of the papilloma virus upon mixture with it *in vitro,* and that sufficient antibody can extravasate into the virus-induced papillomas of wild and domestic rabbits not only to neutralize the virus liberated when the growths are extracted but also to render extracts or suspensions of some growths incapable of eliciting antibody upon injection into normal rabbits. The findings disclose the limitations of immunization-experiments of the sort outlined as a means of demonstrating "masked" virus. Since much antibody is present in the blood of cottontails having cancers deriving naturally from the virus-induced papillomas, and since extravasated antibody has been demonstrated in extracts of the cancers,[7] it follows that the attempted immunization of normal rabbits with extracts of these growths will fail to provide decisive evidence as to whether the virus is or is not present in the malignant tissue, unless the effects of extravasated antibody can first be excluded.

---

[6] Kidd, J. G., Beard, J. W., and Rous, P., *J. Exp. Med.*, 1936, **64**, 63, 79; Kidd, J. G., *J. Exp. Med.*, 1938, **68**, 703, 725, 737.

[7] Kidd, J. G., and Rous, P., *J. Exp. Med.*, 1940, **71**, 469.

11334 P

Demonstration of a Capsule on *Brucella* Cells.

I. Forest Huddleson. (Introduced by W. L. Mallman.)
From the Department of Bacteriology and Hygiene, Michigan State College.

All smooth strains of the 3 species of *Brucella* have been found to possess a definite capsule.

The technic for demonstrating the presence of a capsule on *Brucella* is as follows: A dilute suspension of organisms is prepared in physiological salt solution from a 48-hour liver agar slant growth. A drop of the suspension is drawn across a clean glass slide similar to the method of making a blood film. Allow the film to dry in air. Place a small drop of Kesso India ink near one end of the slide and draw it over the film of organisms by placing the end edge of another slide at such an angle that the India ink spread becomes thinned out. After air drying, the spread is flooded with an aqueous solution of crystal violet (5 cc of saturated alcoholic solution of crystal violet in 95 cc distilled water). The excess of stain is washed off the slide with distilled water at the end of 2 minutes. The stained spreads are dried in air.

When examined under the microscope, magnification 1000×, the organisms appear as dark purple cocci or rods located in the center or near the edge of a clear area surrounded by India ink. The clear area, which is the capsule, varies in size from 2.5 to 4. The size of the capsule varies with the size of the stained cell.

The writer has met with no success in staining the capsule on *Brucella* cells.

The rough form of *Brucella* also shows a capsule. A large proportion of the cells of this form emerge from the capsule when suspended and allowed to stand in distilled water at 37°C. As the cell leaves the capsule, the cell becomes swollen and more intensely stained.

The capsule may be removed from the cell by digestion with hot chloroform-ether mixture acidified with HCl. This treatment also destroys that part of the organism which is made visible by the usual staining methods.

Preliminary studies now in progress in this laboratory show that the capsular material is composed of lipids in close combination with a polysaccharide.

Capsular swelling has not been demonstrated when organisms are incubated with specific serum.

11335

Comparative Study on Bacteriostatic Action of Sulfanilamide, Sulfapyridine, Sulfanilyl-Sulfanilamide and Sulfathiazol upon Enterococci.

ERWIN NETER.

From the Bacteriological Laboratories, Children's Hospital and University of Buffalo, School of Medicine, Buffalo, New York.

At the present time it is not as yet possible to predict from the chemical composition of various sulfanilamide derivatives their chemotherapeutic activity toward different microörganisms that are more or less susceptible to the action of sulfanilamide. Thus, it is necessary to test the efficacy of new compounds both *in vitro* and *in vivo*. It is generally agreed that sulfanilamide in therapeutic doses has little or no effect on enterococci. Recently Helmholz[1] reported that sulfathiazol and sulfamethylthiazol in urine are bactericidal toward enterococci. Hill[2] found that sulfanilamide, sulfapyridine and sulfathiazol in a concentration of 100 mg % have a marked bacteriostatic or germicidal action upon relatively small numbers of *alpha-Streptococcus fecalis* in urine. This author made the important observation that sulfathiazol as present in urine after administration of the drug exerts greater bacteriostatic activity than the same amount added to normal voided urine. Long and Bliss[3] observed that sulfanilamide, sulfapyridine and sulfathiazol in concentrations of 10 mg % only slightly inhibited the growth of hemolytic streptococcus zymogenes (Group D) in beef infusion, 2% Neopeptone, 0.075% dextrose broth. Clinically, Pool and Cook[4] reported sterilization of urine of patients harboring *Streptococcus fecalis* following the administration of sulfathiazol and sulfamethylthiazol, respectively. The concentration of the drug in the urine of these cases exceeded 150 mg %. The following *in vitro* experiments were carried out in order to determine the relative bacteriostatic activity of various sulfanilamide derivatives toward hemolytic and non-hemolytic enterococci.

As culture medium ¼% maltose phenol red broth base (Difco), containing tryptose (1%), sodium chloride (0.5%), dipotassium phosphate (0.1%), and phenol red, was used. Sulfanilamide, sulfapyridine, sulfanilyl-sulfanilamide (Disulon), sulfathiazol (2-sulfanilamidothiazol) and sulfamethylthiazol (2-sulfanilamidomethylthiazol) were added to this culture medium. The drugs were dissolved by heating the broths in a water bath. Then, the broths were tubed and autoclaved at 15 lb pressure for 12 minutes. It may be mentioned that a change of the pH to the acid side occurred when sulfanilyl-sulfanilamide, sulfthiazol and sulfamethylthiazol were dissolved in the broth.

---

[1] Helmholz, H. F., *Proc. Staff Meetings Mayo Clinic*, 1940, **15**, 65.

[2] Hill, J. H., *J. Urol.*, 1940, **43**, 491.

[3] Long, P. H., and Bliss, E. A., PROC. SOC. EXP. BIOL. AND. MED., 1940, **43**, 324.

[4] Pool, T. L., and Cook, E. N., *Proc. Staff Meetings Mayo Clinic*, 1940, **15**, 113.

The strains of hemolytic and non-hemolytic enterococci were the same as those previously used in a study on the bacteriostatic action of sulfanilamide upon members of the enterococcus group.[5] In addition to these, several strains were obtained through the courtesy of Dr. H. F. Helmholz, the Mayo Clinic, Rochester, Minnesota. The strains were cultured in brain-heart infusion broth for 18-24 hours; this culture medium was also used for diluting purposes.

Table I presents an experiment in which the bacteriostatic action of various sulfanilamide derivatives in a concentration of 100 mg % upon a strain of hemolytic enterococcus (dilution of 1:125,000) was tested both at 37°C and 43°C. It may be seen from this table, that: (1) at 37°C, sulfanilamide and sulfapyridine lacked growth inhibitory action, whereas sulfanilyl-sulfanilamide and sulfathiazol delayed the growth of the microörganisms for a short period only. (2) At 43°C, sulfanilamide failed to inhibit the growth of the enterococcus; sulfapyridine delayed its growth, and sulfanilyl-sulfanila-

TABLE I.

Bacteriostatic Action of 100 mg % of Sulfanilamide, Sulfapyridine, Sulfanilyl-sulfanilamide and Sulfathiazol upon a Strain of *Enterococcus hemolyticus* in ¼% Maltose Phenol Red Broth.

		1	2	3	4	5
	Hr. of Incubation	Control broth	Sulfanilamide broth	Sulfapyridine broth	Sulfanilyl-sulfanilamide broth	Sulfathiazol broth
			Incubation at 37°C.			
1.	10	++	+	++	—	—
2.	18	++++	++++	++++	+++	++++
3.	24	++++	++++	++++	++++	++++
4.	48	++++	++++	++++	++++	++++
5.	72	++++	++++	++++	++++	++++
6.	120	++++	++++	++++	++++	++++
			Incubation at 43°C.			
1.	10	—	—	—	—	—
2.	18	+++	+++	—	—	—
3.	24	+++	+++	+++	—	—
4.	48	+++	+++	+++	—	—
5.	72	+++	+++	+++	—	—
6.	120	+++	+++	+++	—	—

— = No visible growth.
+ to ++++ = various degrees of growth.

---

[5] Neter, E., Proc. Soc. Exp. Biol. and Med., 1940, **43**, 52.

mide and sulfathiazol prevented visible growth for 5 days. (3) The growth of the enterococcus in the control broth was slightly retarded and inhibited at 43°C in comparison to that obtained at 37°C.

As in the case of sulfanilamide, the degree of bacteriostasis exerted by sulfanilyl-sulfanilamide and sulfathiazol depends, besides on other factors, upon the size of the inoculum; *e. g.*, in one particular experiment sulfathiazol in a concentration of 0.1% did not delay the growth of a hemolytic enterococcus when 0.1 cc of a 1:50 diluted broth culture was used for inoculation; with a 1:2500 dilution, it delayed the growth for 18 hours and with a 1:6,000,000 dilution it completely prevented visible growth for 8 days. Essentially the same results were obtained with hemolytic and non-hemolytic enterococci.

In preliminary experiments with sulfamethylthiazol it was found that this drug in concentrations of 0.1% or in saturated solution is bacteriostatic toward members of the enterococcus group and is more effective than sulfanilamide in equal concentrations (0.1% to 0.2%).

Previously, it was shown[5] that sulfanilamide in concentration of 1% may completely prevent visible growth of suitable numbers of enterococci in broth incubated at approximately 43°C. In order to further evaluate the relative efficacy of sulfanilamide and its derivatives, the bacteriostatic effect of 1% sulfanilamide was compared with that of sulfanilyl-sulfanilamide, sulfathiazol and sulfamethylthiazol in 0.2% concentration or saturated solution, respectively.

These experiments were carried out with hemolytic and non-hemolytic enterococci. It was found that 1% sulfanilamide is of greater effectiveness than are sulfanilyl-sulfanilamide, sulfathiazol and sulfamethylthiazol in the above mentioned concentrations.

In conclusion: (1) Sulfanilyl-sulfanilamide, sulfathiazol and sulfamethylthiazol in concentrations of 100 mg % or above exert definite bacteriostatic activity toward small numbers of both hemolytic and non-hemolytic enterococci in ¼% maltose broth at 43°C. (2) The growth inhibitory effect of these drugs is greater than that of equal concentrations (0.1% to 0.2%) of sulfanilamide and sulfapyridine. (3) The bacteriostatic effect of sulfanilamide and its derivatives upon the growth of enterococci is greater at 43°C than at 37°C.

We wish to express our appreciation to Alba Pharmaceutical Company for the supply of sulfanilyl-sulfanilamide (Disulon); to Merck & Company for sulfapyridine; to E. R. Squibb & Sons for sulfathiazol; and to Winthrop Chemical Company for sulfamethylthiazol.

11336 P

Acetylmethylcarbinol Enzyme-System of *Aerobacter aërogenes.*

M. Silverman and C. H. Werkman.

From the Bacteriology Section, Industrial Science Research Institute, Iowa State College, Ames, Iowa.

It is generally accepted that in the formation of acetylmethylcarbinol from pyruvic acid by such bacteria as *Aerobacter aërogenes,* the acid must be first decarboxylated to acetaldehyde. No direct evidence, however, has been presented that *Aerobacter* can bring about such a decarboxylation. The difficulty has been due, in part, to the simultaneous activity of hydroclastic enzymes, *i. e.,* enzymes splitting pyruvic acid into formic and acetic acids (*cf.* equation 1). The formic acid is cleaved into H_2 and CO_2. The formation of CO_2 by this reaction does not involve intermediary acetaldehyde.

Employing the technic of Wiggert, *et al.,*[1] an enzyme-preparation has been extracted from cells of *Aerobacter aërogenes,* with apparently no hydroclastic activity, nevertheless, capable of converting pyruvic acid into CO_2 and acetylmethylcarbinol. Typical fermentation-balances are shown in Table I. The ratio of pyruvate utilized to CO_2 and acetylmethylcarbinol formed is approximately 2:2:1.

Attempts to increase the carbinol yield by the addition of acetaldehyde were unsuccessful. The added aldehyde was recovered unchanged. If acetaldehyde is an intermediate, only the biologically nascent form is utilized by this enzyme-preparation.

As indicated in Table II no appreciable production of CO_2 and consequently no carbinol occurs at pH levels above 6.8. There is a direct relationship between CO_2 and carbinol production.

The relationship between pH and the direct cleavage of CO_2 from

TABLE I.
Dissimilation of Pyruvic Acid by Enzyme-preparation of *A. aërogenes.* Values in millimoles.

Initial pyruvic acid	Final pyruvic acid	Pyruvic acid utilized	CO_2	Acetylmethyl-carbinol
9.17	1.60	7.57	7.63	3.30
9.17	1.69	7.48	7.42	3.40
4.99	1.08	3.91	3.62	1.61
2.49	0.16	2.33	—	1.09

3 ml enzyme preparation-vol. 30 ml-5 ml. M/15 KH_2PO_4-atmosphere N_2-30°C.

---

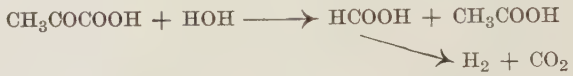
[1] Wiggert, W. P., Silverman, M., Utter, M. F., and Werkman, C. H., *Iowa State Coll. J. Sci.,* 1940, **14**, 179.

TABLE II.
Relationship Between pH and Rate of CO_2 Formation from Pyruvic Acid by Enzyme-preparation of *A. aërogenes*.

pH	3.5	5.6	6.0	6.4	6.8	7.2	7.6	8.0
mm^3 CO_2 in 15 min.	170	122	119	76	25	11	4	9
V.P. test	4+	4+	4+	4+	2+	?	—	—

.15 ml juice-vol. 2.0 ml-1.8 mg pyruvic acid-1 ml PO_4 buffer M/15-atmosphere air.

pyruvic acid by *Aerobacter* will assist in elucidating the mechanism of pyruvate-breakdown. From the investigations of Mickelson and Werkman[2] and from Table II, the carbinol is not formed at alkaline reactions and the predominating mechanism involves reaction I. Under acid conditions both reactions occur. Mickelson[3] has shown that the products of the alkaline fermentation of pyruvate by *Aerobacter* are acetic and formic acids in equimolar proportions.

(1)


$$CH_3COCOOH + HOH \longrightarrow HCOOH + CH_3COOH$$
$$HCOOH \longrightarrow H_2 + CO_2$$

$$(2)\quad 2\ CH_3COCOOH \longrightarrow 2\ CO_2 + CH_3COCHOHCH_3$$

These results may be checked manometrically with cell suspensions as shown in Table III. As the system is made alkaline, more H_2 should be evolved as reaction 2 becomes inhibited. At pH 8.0 the CO_2/H_2 ratio closely approaches the theoretical value of 1.0.

The presence of inorganic phosphate has been found to be essential for pyruvate-breakdown by this enzyme-preparation.

TABLE III.
pH and the Gas Ratios of *A. aërogenes* (whole cells) on pyruvate.

pH	4.5	5.6	6.8	8.0
mm^3 H_2	164	208	335	370
mm^3 CO_2	426	449	445	398
CO_2/H_2	2.60	2.16	1.33	1.08

Vol. 2.3 ml-atmosphere N_2-cell 24 hours old-2.2 mg pyruvic acid.

---

[2] Mickelson, Milo, and Werkman, C. H., *J. Bact.*, 1938, **36**, 67.

[3] Mickelson, Milo, unpublished thesis, Iowa State College, 1939.

AUTHORS' INDEX

(The numerals indicate the page.)

Abramson, H. A. Skin, ultraviolet light, histamine, allergic reactions, 410.
Abt, A. F., Farmer, C. J., and Topper, Y. J. Gastrointestinal absorption, ascorbic acid, 24.
Adkinson, J. L. 225.
Aggeler, P. M., Lucia, S. P., and Goldman, L. Vit. K, synthetic, prothrombin, 689.
Alfredson, B. V., and Sykes, J. F. Bovine electrocardiogram, bundle branch block, 580, 575.
Allen, D. 212.
Allinson, M. J. C. Creatinine, bacterial enzymes, 736.
Anderson, E., and Herring, V. V. Desoxycorticosterone, body weight, 363.
Ansbacher, S., Fernholz, E., and Dolliver, M. A. Vitamin K, water soluble, 652.
Arnow, L. E., Opsahl, J. C., and Watson, C. J. Glutamic acid, from proteins in leukemia, 766, 767.
Aronson, J. D., and Meranze, D. R. Tuberculous lesions, syphilis, 83.

Bachrach, W. H., 36.
Barlow, O. W., and Homburger, E. Thiazol, sulfanilamide, 317.
Bartlett, J. W. 183.
Beach, E. F. 104.
Beard, D. 1, 648, 650.
Beard, J. W. 380, 648, 650.
Beck, F. F. Bile analysis, 603.
Beckman, H., and Ota, R. K. Blood, canary, 268.
Bender, M. B. Parasympathomimetic effects, erythrocytes, 539.
Berkman, S. 434.
Berman, A. L., Snapp, E., and Ivy, A. C. Bile, keto-reacting substances, 547.
Bierman, W. 277.
Biskind, G. R. Methyl testosterone, inactivation, 259.
Blattner, R. J. 255.
Bliss, A. F. *Rana pipiens*, development and trypsin, 769.
Bliss, E. A., and Ott, E. Sulfapyridine, staph. 706, 325.
Blount, R. F. Renal corpuscles, variation in hypophyseal tissue, 537.
Bodian, D. 718.
Bonanto, M. V. 678.
Boroff, D. A. Streptococcal proteins, oil droplets, 294.
Borson, H. J., and Mettier, S. R. Anemia, Vitamin B_6, 430.
Boyce, F. F. 446.
Boyd, E. M., and Gibson, J. D. Ant. pituitary, Brunn reaction, 572.
Boyden, E. A. 534.
Bradford, W. L., and Slavin, B. Pertussis, nasopharyngeal cultures, 590.
Brogdon, E. 629.
Brooks, D. J. 458.
Bronfenbrenner, J., and Kalmanson, G. M. Bacteriophage, antigenic properties, 16.
Brues, A. M. 737.
Brunschwig, A., and Schmitz, R. L. Chloride ion, blood, gastric juice, 438.
Bryan, W. L., and Mason, K. E. Mammary carcinoma, vitamin E, 375.
Bryan, W. R., and Beard, J. W. Papilloma protein, vaccinia virus, 380.
Buchholz, A. M. Antisera, kidney, 20.
Bueding, E. 279.
Bugher, J. C. Virus, micromortar, 422.
Bukantz, S. C. Pneumococcus, capsular polysaccharide, 553; and Bullowa, J. G. M. Pneumococcal capsular polysaccharide, 418.
Bullowa, J. G. M. 418.
Burns, E. L., and Schenken, J. R. Carcinoma, mammary gland, estrogen, 608.
Burrill, M. W. 32, 382.
Burstein, C. L., and Marangoni, B. A. Procaine, ventricular fibrillation, 210.

Cantarow, A. 335.
Chadwick, C. S. Pituitary, ant., extract, water drive, 509.
Chaffee, E. 487.
Chaikoff, I. L., and Connor, C. L. Liver, cirrhosis, fat diet, 638; 349.
Chang, H. S., Hsieh, W. M., Lee, L. Y., and Li, T. H. Acetylcholine, retina, 140.
Chargaff, E. 740.
Charles, D. R., and Rawles, M. E. Tyrosinase, 55.
Cheyne, V. D. Dental caries, fluorine, 58.
Cinberg, B. L., and Goldman, S. F. Chorionic gonadotropic preparations, potency, 208.
Clark, W. G., Arnow, L. E., and Opsahl, J. C. Glutamic acid, embryo tissue proteins, 767.
Claude, A. Leech extracts, "spreading," mucolytic activity, 684
Claus, P. E. 272.
Clifton, C. E. *Clostridium botulinum*, serine, 589; Pyruvic acid, *Clostridium botulinum*, 585.
Climenko, D. R. Sulfanilamide, tuberculosis, 624; and Schmidt, R. L. Sulfanilamide, H. strep., 622.
Cohen, H. R., and Freda, V. C. Antiserum, placental protein, 22; and Nedzel, A. J. Antiserum, placental protein, pregnancy, 249.
Cohen, M. A. 611.
Cole, H. H. 432.
Connor, C. L. 638.
Cook, G. M. 85.
Cooke, J. V., and Blattner, R. J. Staining, virus lesions, 255.
Cooper, F. B., Gross, P., and Lewis, M. Chemotherapy, N^4-n-acylsulfanilylhydroxamides, 491.
Corcoran, A. C. 722.
Couteaux, R., and Nachmansohn, D. Choline esterase, sciatic nerve section, 177.
Crede, R. H., and Moon, H. D. Adrenocorticotropic hormone, thymus, 44.
Culmer, C. U. 225.
Curnen, E. C. 461.

Dalton, A. J., and Masson, G. Adrenalectomy, blood count, 370.
Darrow, C. W. 236.
Davis, H. A. Circulatory failure, NaCl, 354. Dehydration shock, 357.
Davis, H. P. 183.
Dawson, R. F., and Robson, J. M. Fetal placental circulation, 758.
DeGowin, E. L. 251.
Dienes, L. L type colonies, origin, 703.
Dolliver, M. A. 652.
Dorfman, A., Koser, S. A., Horwitt, M. K., Berkman, S., and Saunders, F. Dysentery bacillus, nicotinamide, 434; 391.
Dorfman, R. I. Strophanthin, adrenalectomy, 489.
Doubilet, H., and Bierman, W. Hyperpyrexia, bile duct, cardiac sphincters, 277.

Dragstedt, C. A., Gray, J. S., Lawton, A. H., and Ramirez de Arellano, M. Gastric secretion, histamine, alcohol, 26; Ramirez de Arellano, M., and Lawton, A. H. Peptone, histamine metabolism, 28; 360.
Dreguss, M. 100.
Drill, V. A., and Hays, H. W. Hyperthyroid, vitamin B, 450.

Edgerton, H. E. 455.
Edwards, J. C. 11.
Edwards, L. B. 328.
Eisele, C. W. 308.
Elliott, M. C., Isaacs, B., and Ivy, A. C. Prothrombin, vitamins, 240.
Elman, R. Serum albumin regeneration, amino acids, 14.
Elvehjem, C. A. 161.
Enders, J. F., and Hammon, W. D. Immunization, panleucopenia virus, 194.
Entenman, C. 349.

Farbman, A. A. 181.
Farmer, C. J. 24.
Fernholz, E. 652.
Findley, T., Jr. 11.
Fine, J., Fuchs, F., and Mark, J. Desoxycorticosterone, intestinal obstruction, 514.
Finkelstein, H. 648, 650.
Flynn, J. G., and Warner, E. D. Prothrombin, vitamin K, 190.
Foote, C. L. Sex hormones, gonads, 519.
Forbes, H. S., and Nason, G. I. Metrazol, cerebral vessels, 762.
Forbes, J. C., Outhouse, E. L., and Leach, B. E. Serum esterase, foods, 523.
Fouts, P. J. 722.
Francis, T., Jr. Influenza virus, intranasal inoculation, 337.
Fraser, A. W. 543.
Freda, V. A. 22.
Freedman, H. 212.
Freiman, D. G. 397.
Freund, J., and Bonanto, M. V. Antitoxin, diphtheria, 678.
Friedberg, L. 245.
Friedemann, T. E. Carbohydrate metabolism, bacteria, 148.
Friedman, M. H. F., Saltzstein, H. C., and Farbman, A. A. Gastric secretion, urine, 181.
Fuchs, F. 514.

Geiling, E. M. K. 407.
Geist, S. H., Walter, R. I., and Salmon, U. J. Estrogen, local reaction to implantation, 712, 424.
Gellhorn, E., Darrow, C. W., and Yesinick, L. Blood pressure, nervous system, 236.
Gengerelli, J. A. 64.
Gibbs, G. E. 349.
Gibson, J. D. 572.
Gilson, A. S. 256.
Glaser, R. W. Nematode parasite, bacteria-free culture, 512.
Goettsch, M. 313.
Golden, T. S. 74.
Goldman, L. 689.
Goldman, S. F., 208.
Goss, H. 432.
Gray, J. S., and Bachrach, W. H. Atropine, gastric secretion, alcohol, 36; Culmer, C. U., Wieczorowski, E., and Adkison, J. L. Pyrogen-free urogastrone, 225; 26.
Greenberg, L. D., and Rinehart, J. F. Sulfhydryl, pyruvic acid, yeast, 495.
Greenberg, S. S. 397.
Green, H. Sulfapyridine, tubercle bacillus, 344.
Greene, R. R., and Burrill, M. W. Desoxycorticosterone acetate, androgenic activity, 382; Burrill, M. W., and Ivy, A. C. Estrogen, hypospadias, 32.
Gross, P. 491.
Gunnison, J. B. 672.
Guyer, M. F., and Claus, P. E. Carcinoma, colchicine, 272.
Gyorgy, P., and Rose, C. W. Vitamin H, egg-white injury, 73.

Hadley, P. 102.
Hadley, R. P., and Hadley P. Peroxide, streptococci, 102.
Hall, H. 532, 616.
Hall, R. P., and Loefer, J. B. Culture filtrates, growth, 128.
Hammon, W. D. 194.
Hansen, A. E., Palmer, L. S., and Nelson, J. W. Bone, osteoporosis, hepatoma, 206.
Hansen, W. 46.
Happold, F. C. *C. diphtheriae*, toxin production, 412.
Harrow, B. 468.
Hart, E. B. 161.
Haselwood, L. A. 517.
Hastings, A. B., and Eisele, C. W. Acid-base balance, variation, 308.
Hathaway, I. L. 183.
Haury, V. G., and Cantarow, A. Magnesium sulfate, serum, peritoneal fluid, 335.
Haviland, J. W. 328.
Hays, H. W. 450.
Hechter, O., Levine, R., and Soskin, S. Zinc content, insulin, 361.
Heidelberger, M. 646.
Heinbecker, P. 8.
Hellebrandt, F. A., Rork, R., and Brogdon, E. Gelatin, anaerobic work, 629.
Helmer, O. M. 722.
Herring, V. V. 363.
Hildebrand, G. J. 49.
Hill, F. C. 282.
Hilles, C. 288.
Hoagland, C. L. 757.
Hoffman, W. S. Cholesterol, Liebermann-Burchard reaction, 38.
Homburger, E. 317.
Horwitt, M. K. 434.
Hoskins W., and Webster, B. Androgens, castrate men, 604.
Hou, H. C. Thyroid enlargement, liver feeding, 753.
Howe, H. A., and Bodian, D. Poliomyelitis, entry, 718.
Hoyt, A., and Warner, D. Rabies, vaccine, urea-treated virus, 154.
Hsieh, W. M. 140.
Hsu, C. L. 744.
Hubbard, R. S. 265.
Huddleson, I. F. Brucella, capsule, 773.
Hurd, F. W. 172.

Irvine, V. C. X-radiation, growth, 453.
Isaacs, B. 240.
Isaacs, R. 551.
Ivy, A. C. 32, 240, 547.

Jacobs, J. L. Allergy, skin reactions, 641; Golden, T. S., and Kelley, J. J. Allergy anhydrides, 74.
Janes, R. G., and Nelson, W. O. Stilboestrol, carbohydrate metabolism, 340.
Jennison, M. W., and Edgerton, H. E. Infection, droplet, air, 455.
Jermsta, J. 172.
Jones, E. E. 157.
Jorgensen, M. N., and Hill, F. C. Potassium, intestinal obstruction, 282.
Julianelle, L. A. Trachoma, inclusion body, pathogenesis, 507.
Jungeblut, C. W. Diphtheric toxin, rat, 479; Poliomyelitis, hypnotic agents, 464.

Kalmanson, G. M. 16.
Kamin, H. 468.
Kark, R., Lozner, E. L., and Meiklejohn, A. P. Vitamin B_6 hydrochloride, nutrition, 97.
Katz, L. N. 245.
Keefer, C. S. 664.
Keil, J. H. Spinal cord regeneration, 175.
Keller, A. D. Infundibular nerve fibers, diabetes, 61.
Kelley, J. J. 74.
Kempf, A. H. 705; and Nungester, W. J. Pneumonia, production, **627.**
Kempf, G. F. 722.
Kempf, J. E., and Soule, M. H. Poliomyelitis, extraneural tissues, 476.
Keresztesy, J. C. 118.
Keys, A. Potassium, exercise, 395.
Kidd, J. G. Rabbit papilloma extracts, antibody, 770.
Kligler, I. J., and Olitsky, P. K. Encephalomyelitis, avian cultivation, **680.**
Kohlstaedt, K. G., and Page, I. H. Renin, renal artery constriction, 136, 722.
Koones, H. F. 118.
Kornblum, K. 305.
Koser, S. A., Dorfman, A., and Saunders, F. Pyridine, dysentery bacilli, 391; 434.
Kountz, W. B. 256.
Kremen, A. J., Taylor, H. L., and Hall, H. Skin sensitivity, bovine plasma, 532; **616.**
Kroll, H., Strauss, S. F., and Necheles, H. Dye, abscesses, 228.
Krueger, A. P., and Scribner, E. J. Phage, iodoacetic acid, 416.
Kuntz, A., and Haselwood, L. A. Vasomotor reflexes, 517.

Landsteiner, K., and Wiener, A. S. Agglutinable factor, blood, immune sera, 223.
Last, J. H. 407.
Laszlo, D. 558.
Lavin, G. I., Hoagland, C. L., and Ward, S. M. Cytochrome C, spectrum, 757.
Law, L. W., and Lewisohn, M. Lymphomatosis, 143.
Lawrence, C. A. Sulfanilamide, bacteria, 92.
Lawton, A. H. 26, 28, 360.
Layne, J. A., and Boyden, E. A. Gall bladder, anemia, 534.
Leach, B. E. 523.
Lee, K., and Wu, H. Antibody, Type I pneumococcus, 65.
Lee, L. Y. 140.
Leidenheimer, H., Jr., and Boyce, F. F. Serum volume index, newborn, 446.
Leo, S. 696.
Leuchtenberger, C. 558.
Leuchtenberger, R. 558.
Levine, R. 361.
Lewis, H. 696.
Lewis, M. 491.
Lewisohn, M. 143.
Lewisohn, R., Leuchtenberger, C., Leuchtenberger, R., and Laszlo, D. Adenocarcinoma, yeast extract, 558.
Li, R. C. Gonadotropic potency, pituitary, tryptic digestion, 598.
Li, T. H. 140.
Limarzi, L. R. 29.
Lin, H. A. C., and Isaacs, R. Neutropenia, potassium dicarboxybenzanthracene, 551.
Lipp, W. F., and Hubbard, R. S. Blood storage, 265.
Liu, S. C., and Wu, H. Antigen, pneumococcus, 747.
Locke, A., and Cohen, M. A. Arthritis, rheumatoid, CO_2, **611.**
Loefer, J. B. 128.
Loeffel, E. 212.
Loken, R. 64.
Long, P. H., and Bliss, E. A. Sulfathiazole, pneumococcus, 325; Haviland, J. W., and Edwards, L. B. Sulfathiazole, toxicity, 328.
Lorber, V. Lymph flow, pulmonary edema, 170.
Lozner, E. L. 97.
Lucia, S. P. 689.
Luxen, M. P. 672.
Lynch, C. J. Tumor nodules, lung, 186.

McKee, C. M. 332, 561.
McRary, W. L. 564.
MacBryde, C. M., Freedman, H., Loeffel, E., and Allen, D. Estrogen, stilbestrol, 212.
Macht, D. I., Sherman, S., and Brooks, D. J. Venom, blood, 458.
MacLeod, C. M., Mirick, G. S., and Curnen, E. C. Soil bacillus, toxicity, 461.
MacPherson, C. P. C., and Heidelberger, M. Immunology, egg albumin, **646.**
Main, E. R., Shinn, L. E., and Mellon, R. R. Sulfanilamide, anticatalase activity, 593.
Mann, L. S., and Welker, W. H. Antisera, malignant tissue, 18.
Marangoni, B. A. 210.
Mark, J. 514.
Marshall, M. S., Gunnison, J. B., and Luxen, M. P. Sterility of biologicals, **672.**
Marsland, D. A., and Rugh, R. Sperm, hydrostatic compression, 141.
Martin, D. W. 660.
Mason, K. E. 375.
Mason, M. F., and Rozzell, J. D. Ammonia, determination, 274.
Masson, G. 370.
Megibow, R. S., Friedberg, L., Rodbard, S., and Katz, L. N. Arterial pressure, ureteral occlusion, 245.
Meiklejohn, A. P. 97.
Mellon, R. R. 593.
Meranze, D. R. 83.
Mertz, E. T., and Owen, C. A. Imidazole, blood clotting, 204.
Mettier, S. R. 430.
Meyer, K., and Chaffee, E. Mucopolysaccharide acid, cornea, 487.
Milhorat, A. T., Weber, F. C., and Toscani, V. Dermatomyositis, 470.
Miller, H. R., and Spiegel, E. A. Sleep, thalamic lesion, 300.
Miller, P. A. 389.
Mirick, G. S. 461.
Mitchell, H. S., and Cook, G. M. Cataract, galactose, exercise, 85.
Mohs, F. E. Estrogens, fibroma, 270.
Molitor, H., and Robinson, H. J. Vitamin K_1, toxicity, 125.
Montgomery, M. L., Entenman, C., Gibbs, G. E., and Chaikoff, I. L. Pancreatic juice, liver fat, 349.
Moon, H. D. Adrenocorticotropic hormone, 42; and Hansen, W. Thyroidectomy, adrenocorticotropic hormone, 46; 44.
Moon, V. H., Kornblum, K., and Morgan, D. R. Irradiation sickness, shock, 305.
Morgan, H. R. Leucopenia, antigenic material, *Eberthella typhosa,* 529.
Morgen, D. R. 305.
Morrow, A. S. Allergy, antigen, chigger, 303.
Mueller, J. H., and Miller, P. A. Tetanus toxin production, 389.
Muller, O. H., and Nickel, W. F. Blood pH, hypertension, 89.

Nachmansohn, D. 177.
Nason, G. I. 762.
Nathanson, I. T., Brues, A. M., and Rawson, R. W. Testosterone thyroid, parathyroid, 737.
Necheles, H. 220, 228.

Nedzel, A. J. 249.
Nelson, E. E. 694.
Nelson, J. B. Vaccinia and variola virus, 110.
Nelson, J. W. 206.
Nelson, W. O. 340.
Neter, E. Sulfanilamide, enterococci, 774; Temperature, sulfanilamide, enterococcus, 52.
Nickel, W. F. 89.
Nixon, E. N. 347.
Nungester, W. J., and Kempf, A. H. Quellung phenomenon, polysaccharide, 705; 627.

Obreshkove, V., and Fraser, A. W. *Daphnia magna*, growth, 543.
Ogden, E., Hildebrand, G. J., and Page, E. W. Blood pressure, ischemia, 49.
Oldham, F. K., Last, J. H., and Geiling, E. M. K. Melanophore-dispersing hormone, cetaceans, 407.
Oleson, J. J., Elvehjem, C. A., and Hart, E. B. Spectacled eye condition, 161.
Olitsky, P. K. Intestine, encephalomyelitis, 296, 680.
Opsahl, J. C. 766, 767.
Ota, R. K. 268.
Ott, E. 706.
Outhouse, E. L. 523.
Owen, C. A. 204.

Page, E. W. 49.
Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. J., Kempf, G. F., and Corcoran, A. C Blood pressure, reduction in hypertension, 722; 136.
Paine, W. G., and Nelson, E. E. Pituitary, post, urine, 694.
Palmer, L. S. 206.
Pang, K. H. Encephalitis, cultivation, 755.
Pappenheimer, A. M., and Goettsch, M. Muscular dystrophy, nerve section, 313.
Park, O., and Woods, L. P. Hemmingsen-Krarup mammalian activity recorder, 366.
Paul, J. T., and Limarzi, L. R. Blood, bone marrow, sulfanilamide, 29.
Pelczar, M. J., Jr., and Porter, J. R. Pantothenic acid, bacterial growth, 151.
Pencharz, R. I., Cole, H. H., and Goss, H. Hypophysectomy, pregnant mare serum, 432.
Pentler, C. F. 258.
Perla, D., Freiman, D. G., Sandberg, M., and Greenberg, S. S. Histamine, shock, desoxycorticosterone acetate, 397.
Phatak, N. M., and Pentler, C. F. Staphylococcus enterotoxin, 258.
Popper, H. Vitamin A, fluorescence microscopy, 133; Vitamin A, liver, 234; and Necheles, H. Enzymes, pancreas damage, 220.
Porter, J. R. 151.
Prinzmetal, M., Lewis, H., and Leo, S. Renal ischemia, hypertension, 696.

Rachmilewitz, M. Fibroblasts, urine, 497.
Rake, G., and McKee, C. M. Sulfathiazole, *Staphylococcus aureus*, 561; McKee, C. M., and Shaffer, M. F. Lymphogranuloma, yolk-sac, 332.
Ralli, E. P., and Rubin, S. H. Liver, fatty infiltration, pancreas, 601; and Sherry, S. Hormone, insulin, vitamin C, 669.
Ramirez de Arellano, M., Lawton, A. H., and Dragstedt, C. A. Histamine, trypsin, 360; 26, 28.
Rammelkamp, C. M., and Keefer, C. S. Sulfathiazole, *Staphylococcus aureus*, 664.
Rawles, M. E. 55.
Rawson, R. W. 737.
Reece, R. P., Bartlett, J. W., Hathaway, I. L., and Davis, H. P. Lactation, hormonal inhibition, 183.
Reedman, E. J., Sampson, W. L., and Unna, K. Vitamin B_6, 112.
Reiner, L. 80.
Reinhardt, W. O. Thymectomy, 732.
Rinehart, J. F. 495.
Rinfret, A. Streptococci, capsular swelling, 700.
Robinson, H. J. 125.
Robson, J. M. 758.
Rodbard, S. 245.
Rogoff, J. M., and Nixon, E. N. Adrenal glands, insulin hypoglycemia, 347.
Rolf, D. 1.
Rork, R. 629.
Rose, C. S. 73.
Rosenthal, L. Sulfanilamide, bacilli, 78.
Rozzell, J. D. 274.
Rubin, S. H. 601.
Rugh, R. 141.

Salmon, A. A. 709.
Salmon, U. J., and Salmon, A. A. Genital tract, pregneninolone, 709; Geist, S. H., and Walter, R. I. Estrogenic hormone crystal, implantation, 424, 712.
Saltzstein, H. C. 181.
Sampson, W. L. 112.
Samuels, L. T. 263.
Sandberg, M. 397.
Saunders, F. 391, 434.
Schenken, J. R. 608.
Schmidt, L. H., and Hilles, C. Pneumococcus, sulfapyridine, 288.
Schmidt, R. L. 622.
Schmitz, R. L. 438.
Scott, B. L. Androgen, 216.
Scribner, E. J. 416.
Scudi, J. V., Koones, H. F., and Keresztesy, J. C. Vitamin B_6, urinary excretion, 118.
Searle, D. S., and Reiner, L. Carbon dioxide, glucose metabolism, trypanosome, 80.
Seegers, W. H. 251.
Selye, H. Gonadotropic hormone, 404; Progesterone, estrin, 343.
Shaffer, M. F. 332.
Sharp, D. G., Taylor, A. R., Finkelstein, H., and Beard, J. W. Encephalomyelitis immunization, 650, 648.
Sherman, S. 458.
Sherry, S. 669.
Shinn, L. E. 593.
Shorr, E. Estrogens, vaginal smear, 501.
Silverman, M., and Werkman, C. H. Aerobacter aerogenes, enzymes, 777.
Sklow, J. 656.
Slavin, B. 590.
Smith, G. V. S., and Jones, E. E. Tumor, thymus gland extract, 157.
Smith, J. R., Kountz, W. B., and Gilson, A. S. Heart sound, 256.
Smith, S. G., and Martin, D. W. Cheilosis, vitamin B_6 treatment, 660.
Snapp, E. 547.
Soskin, S. 361.
Soule, M. H. 476.
Spiegel, E. A. 300.
Spiegelman, A. R. Estrogen, insulin, diabetes, 307.
Spink, W. W., Hurd, F. W., and Jermsta, J. Prontosil-soluble, sulfanilamide, 172.
Stekol, J. A. S-benzylglutathione, benzylmercapturic acid, 108.
Stephens, D. J. Cl excretion, hypothyroidism, 742.
Stern, A., and Beach, E. F. Homocystine, microdetermination, 104.
Stevens, B. 616.

Stoesser, A. V. Pneumonia, plasma lipids, 168; Sulfapyridine, plasma lipid, pneumonia, 201.
Strauss, S. F. 228.
Strong, L. C. Cancer, mammary, control, 634.
Sulman, F., and Sklow, J. Prolan, gonadotropic response, 656, 86.
Summers, W. A. *Dirofilaria immitis*, 448.
Suto-Nagy, G. de. Progesterone-like subst. plants, 674.
Sykes, J. F., and Alfredson, B. V. Bovine electrocardiogram, potassium, 575; 580.
Szego, C. M., and Samuels, L. T. Estrone, estimation, 263.

Takacs, W. S. 536.
Taylor, A. R., Sharp, D. G., Finkelstein, H., Beard, D., and Beard, J. W. Encephalomyelitis purification, 648, 650.
Taylor, H. L. 532.
Taylor, R. M. Influenza, virus, immunity response, 541; and Dreguss, M. Influenza virus, 100.
Tocantins, L. C. Prothrombinemia-hypo, 729.
Toomey, J. A., and Takacs, W. S. Poliomyelitis, rats, 536.
Topper, Y. J. 24.
Toscani, V. 470.
T'ung, T., and Hsu, C. L. Pneumococcus, dissociation by radon, 744; and Wong, S. C. *M. tuberculosis*, 70, 749.
Tyler, D. B. 734.

Unna, K. Antidermatitic effect, vitamin B_6, 122; Vitamin B_6, toxicity, 116; 112.

Van der Scheer, J., and Wyckoff, R. W. G. Tetanus antitoxin sera, 427.
Van Harreveld, A., and McRary, W. L. Blood pressure, anoxia, 564.
Volker, J. F. Tooth enamel, fluorosed, solubility, 643.

Wagreich, H., Kamin, H., and Harrow, B. Phenylacetic acid, glucuronic acid, 468.
Walter, R. I. 424, 712.
Wangensteen, O. H., Hall, H., Kremen, A., and Stevens, B. Blood plasma, intravenous, 616.
Ward, S. M. 757.
Warner, D. 154.
Warner, E. D., DeGowin, E. L., and Seegers, W. H. Blood storage, prothrombin, 251; 190.
Watson, C. J. 766.
Weber, F. C. 470.
Webster, B. 604.
Wegria, R. Heart, mammalian ventricle, systole, 261.
Weiss, C. Staphylococcal antitoxin, 441.
Welker, W. H. 18.
Werkman, C. H. 777.
West, H. D. Detoxication of diphenyl, sulfur, 373.
White, H. J. Antibacterial activity, peptone-dextrose broth, 214.
White, H. L., Findley, T., Jr., and Edwards, J. C. Diodrast clearance, 11; and Heinbecker, P. Diodrast clearance, 8; and Rolf, D. Iodine determination, micro method, 1.
Wieczorowski, E. 225.
Wiener, A. S. 223.
Wiener, M. Eye refraction, 384.
Wilmer, H. A. Growth, human body, 545; Skin, growth, surface area, 386.
Wilson, S. J. Antithrombin, estimation, 676.
Wilson, W. E. 279.
Winfield, J. M. Bile, gastric motility, 474.
Wong, S. C. 70; and T'ung, T. Diphtheriae, corynebacterium, sp. protein, 749.
Woods, L. P. 366.
Woolley, D. W. Rat filtrate, factor, 352.
Wortis, H., Bueding, E., and Wilson, W. E. Blood, bisulfite binding substances, 279.
Wu, H. 65, 747.
Wyckoff, R. W. G. 427.

Yesinick, L. 236.
Youngburg, G. E., and Youngburg, M. V. Silicosis, 146.
Youngburg, M. V. 146.

Ziegler, M. R. Plasma electrolytes, insulin, 165.
Ziff, M., and Chargaff, E. Heparin mode of action, 740.
Ziskind, E., Loken, R., and Gengerelli, J. A. Metrazol, learning, 64; and Tyler, D. B. Insulin shock, decerebrate preparations, 734.
Zondek, B. Bleeding, estrogenic hormone, 570; and Sulman, F. Estrogen, vaginal cycle, 86.

SUBJECT INDEX

(*The numerals indicate the page.*)

Abscess, dye, 228.
Acetylcholine, retina, 140.
Acid-base balance, variations, 308.
Agglutinable factor, blood, 223.
Allergy, anhydrides, 74.
  antigen, chigger, 303.
  bovine plasma, 532.
  histamine, light, 410.
  skin reactions, **641.**
Ammonia, micro-determination, 274.
Anemia, gall bladder, 534.
  Vit. B_6, 430.
Anoxia, blood pressure, 564.
Arthritis, rheumatoid, CO_2, **611.**
Atropine, gastric secretion, 36.

Bacteria, aerobacter aerogenes, enzymes, 777.
  anti-activity, peptone-dextrose broth, 214.
  growth, pantothenic acid, 151.
  L type colonies, 703.
Bacteriophage, antigenic properties, 16.
Bile, analysis, 603.
  gastric motility, 474.
  keto-reacting substance, 547.
Blood albumin, amino acids, 14.
  canary, 268.
  cerebrospinal fluid, bisulfite, 279.
  circulatory failure, NaCl, 354.
  count, adrenalectomy, 370.
  pH, hypertension, 89.
  plasma, intravenous, **616.**
  preserved, prothrombin, 251.
  pressure, anoxia, 564.
    ischemia, 49, **696.**
    nervous system, 236.
    ureteral occlusion, 245.
  serum-anti, kidney, 20.
  malignant tissue, 18.
  placental protein, 22.
  serum esterase, foods, 523.
    volume index, newborn, 446.
  storage, 265.
  venom, 458.
Brain, thalamic lesions, sleep, 300.
Brucella, capsule, 773.

Carbohydrate metabolism, bacteria, 148.
Cataract, galactose, 85.
Cerebrospinal fluid, blood, bisulfite, 279.
Cholesterol, Liebermann-Burchard reaction, 38.
Choline esterase, sciatic nerve section, 177.
Clostridium botulinum, pyruvic acid, 585.
  serine, 589.
Colchicine, carcinoma, 272.
Creatinine, bacterial enzymes, 736.
Cytochrome C, spectrum, 757.

Daphnia magna, growth, 543.
Dehydration shock, 357.
Dental caries, fluorine, 58.
  enamel, fluorosed, solubility, **643.**
Dermatomyositis, metabolism, 470.
Diabetes, infundibular nerve fiber, 61.
Diodrast clearance, 8, 11.
Diphenyl detoxication, sulfur, 373.
Diphtheriae, corynebacterium, sp. protein, 749.
  toxin, rat, 479.
  toxin production, 412.
Dirofilaria immitis, 448.
Dysentery bacillus, nicotinamide, 434.
  pyridine, 391.

Electrocardiogram, bovine, bundle branch block, 580.
  bovine, low potassium, 575.
Encephalomyelitis, intestine, 296.
Encephalitis, 755.
Enzymes, pancreas, 220.
Erythrocytes, parasympathomimetic effects, 539.
Eye condition, spectacled, 161.
  refraction, 384.

Fetal placental circulation, 758.
Filtrate factor, 352.
Filtrates, old culture, growth, 128.
Fibroblasts, urine, 497.
Fluorine, dental caries, 58.

Galactose, cataract, 85.
Gall bladder, anemia, 534.
Gastric secretion, atropine, 36.
  histamine, 26.
  urine, 181.
  intestinal absorption, ascorbic acid, 24.
Gelatin, anaerobic work, **629.**
Glucose metabolism, trypanosome, 80.
Glutamic acid, proteins in leukemia, 766.
  embryo tissue proteins, 767.
Glutathione-S-benzyl benzylmercapturic acid, 108.
Growth, human body, 545.
  trypsin, *R. pipiens,* 769.
  x-radiation, 453.

Heart, excitability, systole, 261.
  sound, 256.
  sphincters, hyperpyrexia, 277.
Hemmingsen-Krarup mammalian activity recorder, 366.
Hemorrhagic anti-, compounds, water soluble, 652.
Heparin, 740.
Histamine, allergic reaction, light, 410.
  gastric secretion, 26.
  peptone shock, 28.
  trypsin, 360.
Homocystine, microdetermination, 104.

Hormone, adrenal gland, insulin hypoglycemia, epinephrine, 347.
  Adrenalectomy, blood count, 370.
  Adrenocorticotropic hormone, 42.
    thymus, 44.
    thyroid, 46.
  Androgens, castrate men, **604.**
    percutaneous administration, 216.
  Desoxycorticosterone acetate, androgen, 382.
    acetate, shock, 397.
    body weight, adrenalectomy, 363.
    intestinal obstruction, 514.
  Diabetes, estrogen, 307.
  Estrogen, carcinoma, 608.
    diabetes, 307.
    fibroma, 270.
    implantation, 712.
    induced hypospadias, 32.
    vaginal cycle, 86.
    vaginal smear, 501.
    bleeding, 570.
    crystal, implantation, 424.
    therapy, stilbestrol, 212.
    estimation, 263.
  Gonadotropic hormone, 404.

potency, pituitary, tryptic digestion, 598.
preparations, chorionic, potency, 208.
Hypophysectomy, pregnant mare serum, 432.
Insulin, plasma electrolytes, 168.
shock, decerebrate preparations, 734.
vitamin C, 669.
zinc content, 361.
Pituitary, ant., Brunn reaction, 572.
extract, water drive, 509.
post, urine, 694.
Pregneninolone, gonads, 709.
Progesterone, estrin, 343.
-like subst. plants, 674.
Prolan, gonadotropic response, 656.
Sex, gonads, 519.
Testosterone, methyl, inactivation, 259.
thyroid, parathyroid, 737.
Thymectomy, 732.
Thymus, adrenocorticotropic hormone, 44.
gland extract, tumor, 157.
Thyroid, adrenocorticotropic hormone, 46.
enlargement, liver feeding, 753.
-hyper, vitamin B, 450.
-hypo, Cl excretion, 742.

Hyperpyrexia, bile duct, cardiac sphincters, 277.
Hypertension reduction, 722.
renal ischemia, 696.

Imidazole, blood clotting, 204.
Immunology, egg albumin, 646.
Infection, air, 455.
Intestine, encephalomyelitis, 296.
obstruction, desoxycorticosterone, 514.
obstruction, potassium, 282.
Iodine determination, micro method, 1.

Lactation, inhibition, 183.
Leech extr. "spreading," mucolytic activity, 684.
Leucopenia, antigenic material, *E. typhosa*, 529.
Light, whealing response, 410.
Liver, cirrhosis, fat diet, 638.
fatty infiltration, pancreas, 601.
vitamin A, 234.
Lymph flow, pulmonary edema, 170.

Magnesium sulfate, serum, peritoneal fluid, 335.
Melanophore-dispersing hormone, distribution, 407.
Metabolism, carbohydrate, stilboestrol, 340.
Metrazol, cerebral vessels, 762.
learning, 64.
Micro method, iodine determination, 1.
M. tuberculosis, multiplication, 70.
Mucopolysaccharide acid, cornea, 487.
Muscle, metabolism, gelatin, 629.
dystrophy, nerve section, 313.

Nematode parasite, bacteria-free culture, 512.
Nerve, infundibular, diabetes, 61.
system, blood pressure, 236.
Neutropenia, potassium dicarboxybenzanthracene, 551.
Nicotinamide, dysentery bacillus, 434.

Osteoporosis, hepatoma, 206.

Pancreas, enzymes, 220.
liver fat, insulin, 349.
Pantothenic acid, bacterial growth, 151.
Parasympathomimetic effects, erythrocytes, 539.
Peptone shock, histamine, 28.
Permeability, chloride ion, 438.
Peroxide, streptococci, 102.
Pertussis, nasopharyngeal culture, 590.
Phage, iodoacetic acid, 416.
Phenylacetic acid, glucuronic acid, 468.
Plasma electrolytes, insulin, 165.
Pneumococcus, antibody, acid, 65.
antigen, 747.
dissociation by radon, 744.
III, growth, 553.
polysaccharide, 418.
sulfapyridine, 288.
Pneumonia, plasma lipids, serum, 168.
production, 627.
Potassium, exercise, 395.
Pregnancy, antiserum, placental proteins, 249.
Procaine, ventricular fibrillation, 210.
Prothrombin deficiency, vitamins, 240.
-hypo, 729.
vitamin K, jaundice, 190.
Pyridine, dysentery bacilli, 391.

Quellung phenomenon, polysaccharide, 705.

Rabies, urea-treated virus, 154.
Regeneration, spinal cord, 175.
Renal corpuscles, alteration, 537.
Renin, renal artery constriction, 136.
Retina, acetylcholine, 140.

Shock, dehydration, 357.
desoxycorticosterone acetate, 397.
irradiation sickness, 305.
Silicosis, 146.
Skin, growth, 386.
Soil bacillus, toxicity, 461.
Sperm, hydrostatic compression, 141.
Staining, virus lesions, 255.
Staphylococcus enterotoxin, 258.
Sterility, biologicals, 672.
Streptococci, capsular swelling, 773.
proteins, oil droplets, 294.
Strophanthin, adrenalectomy, 489.
Sulfanilamide, anticatalase activity, 593.
bacilli, 78.
bacteria, thiazol, 92.
blood, bone, 29.
enterococci, 774.
H strep., 622.
N^4-n acylsulfanilylhydroxamide, 491.
prontosil-soluble, 172.
temperature, 52.
thiazol, 317.
tuberculosis, 624.
Sulfapyridine, plasma lipids, pneumonia, 201.
pneumococcus, 288.
staph., 706.
tubercle bacillus, 344.
Sulfathiazole, pneumococcal infection, 325.
staph. aureus, 561, 664. 706.
toxicity, 328.
Sulfhydryl, pyruvic acid, 495.
Syphilis, tuberculous lesion, 83.

Tetanus antitoxin sera, 427.
toxin production, 389.
Thrombin, anti- estimation, 676.
Toxin, anti- diphtheria, 678.
staphylococcal, intramuscular injection, 441.
Trachoma, pathogenesis, 507.
Trypanosome, glucose metabolism, 80.
Tuberculous lesion, syphilis, 83.
Tumor, adenocarcinoma, yeast extract, 558.
carcinoma, colchicine, 272.

mammary gland, estrogen, 608.
vitamin E deficiency, 375.
fibroma, estrogen, 270.
lymphomatosis, 143.
lymphogranuloma venereum, yolk-sac, 332.
mammary, control, 634.
nodules, lungs, heredity, 186.
papilloma extracts, antibody, 770.
papilloma protein, vaccinia virus, 380.
thymus gland extract, 157.
Tyrosinase, 55.

Ureteral occlusion, blood pressure, 245.
Urogastrone, pyrogen-free, 225.

Vasomotor reflexes, 517.
Venom, blood, 458.
Virus, encephalomyelitis, avian, 680.
immunization, 650.
purification, 648.
immunity, 541.
influenza, 100.
intranasal inoculation, 337.
lesions, staining, 255.
micromortar, 422.
panleucopenia, immunization, 194.
poliomyelitis, entry, 718.
extraneural tissues, 476.
hypnotic agents, 464.
rats, 536.
vaccinia, papilloma protein, 380.
variola, 110.
Vitamin A, fluorescence microscopy, 133.
liver, 234.
B, hyperthyroid, 450.
B$_6$, anemia, 430.
antidermatitic effect, 122.
crystalline, 112.
cheilosis, 660.
nutrition, 97.
toxicity, 116.
urinary excretion, 118.
Vitamin C, excretion, insulin, 669.
gastrointestinal absorption, 24.
E deficiency, carcinoma, 375.
H, egg white injury, 73.
K, prothrombin, jaundice, 190.
synthetic, prothrombin, 68
toxicity, 125.
water soluble, 652.
prothrombin deficiency, 240

X-radiation, growth, 453.

√